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PHYSIOLOGIC SPECIALIZATION IN SOME CEREAL SMUTS[1]

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INTRODUCTION

The discovery of the phenomenon of physiologic specialization in the fungi is undoubtedly one of the most important developments in plant pathology. It was first suggested by Schroeter (43), in 1879, and since that time physiologic specialization has been found to be of very wide occurrence among plant pathogenes. Reed (33), in 1918, summarized in detail the information on physiologic specialization in the fungi which was available at that time. From 1879 to 1918 classic work was done on physiologic specialization in the rusts and powdery mildews, but investigations on the phenomenon in the smut fungi seem to have been neglected. Kniep (28), in 1919, was the first to show that physiologic specialization does occur in the smut fungi. He noticed differences in the appearance of the sporidial cultures of *Ustilago violacea* (Pers.) Fuckel from different host plants. Zillig (51), in 1921, then demonstrated that there were physiologic forms of this fungus that could be differentiated on the basis of their ability to infect certain members of the Caryophyllaceae and not others. This work was later confirmed by Liro (32) and Bauch (4).

The earlier investigators distinguished physiologic forms only by pathogenicity. Now, however, physiologically distinct entities within a species can be distinguished on the basis of cultural characters (6, 7, 9), physico-chemical reactions (13, 21, 24, 27), and, in some cases, by differences in morphology (1, 31). Thus, according to Stakman (44), there are four general methods for recognizing physiologic forms: cultural characters, physico-chemical relations, morphology (to a limited extent), and pathogenicity. The study of cultural and physico-chemical differences within a

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species of a fungus is of importance since they may serve as the first indication of pathogenic differences. In fact, as has been pointed out, physiologic specialization in the smut fungi was first discovered by finding differences in cultural characteristics of *U. violacea*. Christensen and Stakman (9) differentiated 15 physiologic forms of *Ustilago zeae* (Beck.) Ung. on the basis of cultural characteristics. At least 7, and possibly 8, of these forms were then differentiated on the basis of their parasitic behavior on 10 selfed lines of corn. Broadfoot (5) distinguished 9 physiologic forms of *Fusarium lini* Bolley by differences in cultural characters. All of these forms were then differentiated by their parasitism on four varieties of flax. Similar results have been obtained by Christensen (8) with *Helminthosporium*

FIG. 1. Pure culture of *Ustilago avenae* obtained from chlamydospores which had been washed previously in a 1 per cent solution of copper sulphate for a period of 27 hours.

sativum P. K. and B. and by Ezekiel (14) with *Sclerotinia americana* (Wormald) Nor. and Ezek. Letcher and Willaman (30) found that the physiologic forms of *F. lini*, which were originally recognized by cultural characters and pathogenicity, differed also in their ability to produce alcohol from sugar.

In the production of disease-resistant varieties of crop plants the study of physiologic specialization in the fungi is of great importance. Varieties which are immune or resistant to certain pathogenes in one locality may be entirely susceptible when grown in another geographical area. In a study of physiologic forms of oat smuts, Reed (35) found striking differences in the reaction of varieties of oats to smuts collected in different geographical areas. *Avena brevis* Roth has always been highly resistant to *Ustilago levis* (Kellerm. and Swingle) Magn. when inoculated with the smut collected in Missouri, but when inoculated with the smut from Wales 100 per cent of the plants became infected. A virulent form was found in the United States, however, which did not occur in Wales. One hundred per cent of the plants of Canadian, a variety of *Avena sativa* L., became infected when inoculated with *U. levis* from Missouri, but the Welsh smut caused no infection whatever. Sampson (41) obtained results similar to those obtained by Reed with physiologic forms of smuts collected in Missouri and in Wales. Similar results have been obtained by Faris (16) on the reaction of varieties of barley to physiologic forms of *Ustilago hordei* (Pers.) K. and S. Tisdale and Griffiths (49) found differences in the reaction of wheat varieties to physiologic forms of *U. tritici* (Pers.) Rost.

The investigations reported in the present paper include a study of the cultural differences and physico-chemical relations between physiologic forms of some of the cereal smut fungi. The investigations also include a study of varietal resistance to smut in wheat, oats, and barley with reference to the pathogenicity of physiologic forms.

SPECIFIC OBJECTS OF THE INVESTIGATION

The objects of the present investigation were:

1. To ascertain the number and distribution of physiologic forms of *Ustilago tritici, U. nuda, U. hordei, U. avenae, U. levis, Tilletia levis,* and *T. tritici.*

2. To ascertain the possible differences in cultural characteristics and the effect of temperature on the growth of physiologic forms of *U. tritici, U. nuda, U. hordei, U. avenae,* and *U. levis.*

3. To ascertain the possible differences in pathogenicity of *T. levis, T. tritici, U. hordei, U. levis,* and *U. avenae.*

4. To determine the varietal resistance of wheats to *T. levis,* and of oats to *U. levis* and *U. avenae.*

CULTURAL DIFFERENCES BETWEEN PHYSIOLOGIC FORMS

During 1925 and 1926 *U. nuda, U. tritici, U. hordei, U. avenae,* and *U. levis* were collected from widely separated localities, as indicated in table 1. Pure cultures of the smuts were often obtained by transferring

TABLE 1.—*Source of physiologic forms of various cereal smuts*

Species	Form	Source of collection	Year	Collector
Ustilago tritici	1	Minnesota	1925	Writer
	2	Egypt		T. Fahmy
	3	New South Wales		R. J. Noble
	4	Italy		E. Pantanelli
	5	Sweden		T. Lindfors
	6	Bulgaria		D. Atanasoff
	7	Belgium		E. Marchal
	8	Hungary		B. Husz
	9	France	1926	J. Dufrenoy
	10	Michigan	1927	J. J. Christensen
	11	Ohio		do
	12	Kansas		H. E. Parson
	13	Pennsylvania		J. J. Christensen
	14	Wisconsin		do
Ustilago nuda	1	Minnesota (St. Paul)	1925	Writer
	2	Manitoba		do
	3	California		F. W. Briggs
	4	Bulgaria		D. Atanasoff
	5	Belgium		E. Marchal
	6	Hungary		B. Husz
	7	Germany	1926	T. E. Roemer
	8	France		J. Dufrenoy
	9	Sweden	1925	T. Lindfors
	10	Italy		E. Pantanelli
	11	Wisconsin	1927	Writer
	12	Minnesota (Farmington)		do
Ustilago hordei	1	Italy	1925	E. Pantanelli
	2	Minnesota (St. Paul)	1927	Writer
	3	Minnesota (St. Paul)		do
	4	Minnesota (Waseca)		do
	5	Minnesota (Chaska)		E. C. Stakman
	6	Kansas		H. E. Parson
	7	Iowa		R. O. Bulger
Ustilago levis	1	Minnesota (St. Paul)	1927	Writer
	2	Minnesota (Crookston)		do
	3	Virginia		R. M. Nelson
	4	Oklahoma		H. E. Parson
	5	China	1925	C. C. Chen

TABLE 1.—*Cont.*

Species	Form	Source of collection	Year	Collector
Ustilago avenae	1	Minnesota (St. Paul)	1926	Writer
	2	Germany		T. E. Roemer
	3	Italy		E. Pantanelli
	4	France		J. Dufrenoy
	5	Oklahoma (Chickasha)	1927	W. Butler
	6	Oklahoma (Ryan)		do
	7	Oklahoma (Perry)		H. E. Parson
	8	Texas (Greenville)		do
	9	Texas (Dallas)		W. Butler
	10	Kansas (Wichita)		H. E. Parson
	11	Kansas (Manhatten)		E. B. Lambert
	12	South Dakota		R. O. Bulger
	13	Sector from Form 12		
	14	Missouri (Elden)		H. E. Parson
	15	Missouri (Sedalia)		do
	16	Wisconsin (Arcadia)		J. J. Christensen
	17	Illinois (Urbana)		do
	18	New York (Ithaca)		do
Tilletia levis	1	Hungary	1925	B. Husz
	2	Minnesota		Writer
	3	Egypt		T. Fahmy
Tilletia tritici	1	New Zealand	1925	G. H. Cunningham
	2	Norway		I. Jorstad

the chlamydospores directly to the culture medium. In other cases chlamydospores were suspended in a 1 per cent solution of copper sulphate for from 12 to 27 hours. Pure cultures then were obtained by transferring the chlamydospores directly to the medium by means of a platinum loop. (See figure 1.) In preliminary tests with several different kinds of media, a 2 per cent potato dextrose agar was found to be the best medium for purposes of studying cultural differences of the physiologic forms of these smuts.

Before final transfers were made for the comparative tests, the pure cultures were grown for a period of two weeks on equal quantities of potato dextrose agar. At the end of this time, with the exception of tests with *U. hordei,* triplicate series of 200 cc. Erlenmeyer flasks containing 35 cc. of 2 per cent potato dextrose agar were inoculated with small, approximately equal portions of each culture. In the comparative tests with *U. hordei,* 150 cc. Erlenmeyer flasks containing 30 cc. of the 2 per cent potato dextrose agar were used. All of the cultures were subjected to the same environ-

mental conditions. The agar was made up in one batch, poured and autoclaved at the same time, and the cultures were grown at room temperature in the laboratory.

The following characters were used in differentiating the forms on culture media: color, topography, character of surface, consistency, and type of margin. The colors were classified according to Ridgway's "Color Standards and Color Nomenclature" (37).

Ustilago tritici. In a preliminary paper, Rodenhiser (39) reported the existence of three physiologic forms of *U. tritici* differentiated on the basis of cultural characteristics. This work has been extended, and 14 distinct forms of *U. tritici,* obtained from the various sources indicated in table 1, have been distinguished on culture media. The data on cultural characteristics of four of these forms are summarized in table 2 and a photograph of them is shown in figure 2. There were striking differences in the cultural characters of the different forms. Many differences in color were observed. For example, form 1 developed an avellaneous color grading to pearl gray in the margin, which may be contrasted with the cartridge-buff to pale olive-gray color of form 10. The cultures of these two forms also differed greatly in topography. Form 1 was umbonate, and it developed fleshy, branched strands which grew in a distinctly counter-clockwise direction. On the other hand, the topography of form 10 was slightly convex, smooth, and scattered over the surface were a few fleshy spines. The surface of the colonies of the different forms ranged from dull to waxy, and the margins from entire to undulating. Under the same environmental conditions the cultural characteristics of any particular form of all the smuts studied were remarkably constant (see figures 7 and 9). Cultural differences were detected, however, only when proper differential materials were used. Dif-

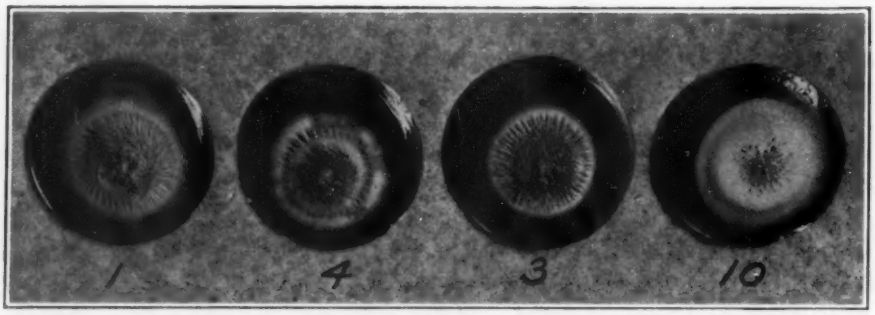

FIG. 2. Four physiologic forms of *Ustilago tritici* grown on potato dextrose agar. Form 1, from Minnesota; form 4, from Italy; form 3, from New South Wales, Australia; form 10, from Michigan.

TABLE 2.—*Cultural characteristics of physiologic forms of Ustilago tritici on potato dextrose agar, 60 days after inoculation*

Source of collection	Form	Color	Topography	Surface	Consistency	Margin
St. Paul, Minnesota Writer	1	Avellaneous to pearl gray in the margin	Umbonate; center covered with fleshy branched strands becoming distinctly counter-clockwise; ray portion smooth	Waxy	Mycelioid and leathery	Entire
New South Wales R. J. Noble	3	Avellaneous to wood brown; margin pearl gray	Convex; complete center coral-like; ray radially furrowed	do	do	do
Italy E. Pantanelli	4	Umbonate portion wood brown grading to light brownish drab; ray pale olive gray	Umbonate with many fine spiney projections; smooth to very slightly furrowed in the ray	Dull	do	Undulating
Williamston, Michigan J. J. Christensen	10	Center cartridge buff; ray pale olive gray	Slightly convex; center smooth with scattered spines	do	do	Entire

ferent forms sometimes looked almost alike on one medium but were entirely different in appearance on another. There also was a great deal of variation in the cultural characters of the same form when grown under different environmental conditions. Similar facts have been observed by Christensen (6 and 7) for *Helminthosporium sativum*, and by Christensen and Stakman (9) for *Ustilago zeae* (Beck.) Ung.

Ustilago nuda. It is evident that there also are distinct physiologic forms of *U. nuda.* Cultures of 12 collections from 12 sources were studied, and all were found to have just as distinct differences in cultural characteristics as those described for *U. tritici.* Cultural characteristics of six of these forms were reported by the writer (39) in a preliminary paper. A detailed description of four of the forms is given in table 3 and a photograph of three forms is shown in figure 3.

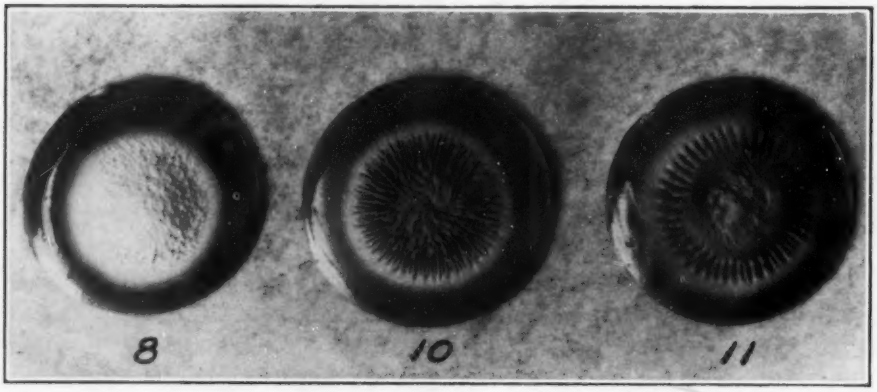

FIG. 3. Three physiologic forms of *U. nuda* grown on potato dextrose agar. Form 8, from France; form 10, from Italy; form 11, from Wisconsin.

The differences in cultural characteristics of different forms of *U. nuda* are sometimes far greater than those between the so-called species *U. tritici* and *U. nuda.* In fact, when grown at 30° C., there is a distinct similarity between the culture of form 6 of *U. nuda* obtained from material collected in Hungary and that of form 2 of *U. tritici* from Egypt (Fig. 4).

All of the morphologic structures of *U. tritici* formed in culture and described by Sartoris (42) were noted. Chlamydospores of both smuts developed in culture. Riehm (38) states that the mycelium of *U. nuda* formed in culture produces straight lateral branches at right angles to the main axis, while in *U. tritici* the lateral branches are curved and more variable in their insertion. The writer, however, observed no consistent differences, such as those described by Riehm. Within the physiologic forms

TABLE 3.—*Cultural characteristics of physiologic forms of Ustilago nuda on potato dextrose agar, 60 days after inoculation*

Source of collection	Form	Color	Topography	Surface	Consistency	Margin
St. Paul, Minnesota Writer	1	Light pinkish cinnamon, with sorghum-brown and smoke-gray patches	Umbonate; slightly felty, with tendency to be smooth at margin; few radial ridges in margin	Chalky	Mycelioid and leathery	Lobate and mycelioid
France J. Dufrenoy	8	Light buff; margin ivory yellow	Umbonate; distinctly felty, margin slightly striated counter-clockwise	Dull to china-like	do	Entire and mycelioid
Italy E. Pantanelli	10	Vinaceous buff; margin pallid neutral gray	Raised; high radial ridges gradually disappearing near the margin	Waxy	do	do
Wisconsin Writer	11	Wood brown, becoming tilleul buff at the margin	Convex; center slightly felty with distinct counter-clockwise striations, uniformly deeply furrowed	Waxy to china-like	do	do

of both species all gradations of these morphologic characters were observed. In fact, by changing the environment in which forms of either species are growing, the entire morphological character of the growth may be changed. These variations were brought about by changing the consistency of the culture medium and the kind of medium, and by growing the cultures at different temperatures.

Counter-clockwise growth of mycelium is characteristic of some of the forms at certain temperatures. A study was made of the effect of temperature on this type of growth in four physiologic forms of *U. nuda*, and the

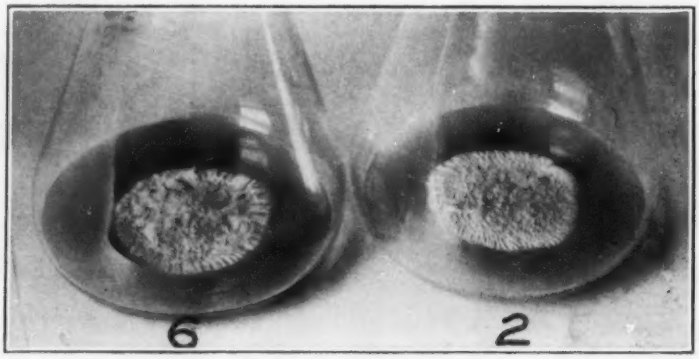

FIG. 4. Twenty-one-day-old cultures of *U. nuda* form 6 and *U. tritici* form 2, grown on potato dextrose agar at 30° C. Note the similarity.

data are summarized in table 4. Counter-clockwise growth of mycelium was very conspicuous when the cultures of form 1 were grown at 15° C., but slightly less so at 20° C. At 10°, 25°, and 30° C., however, the mycelium did not grow in a counter-clockwise manner. No counter-clockwise growth developed in cultures of form 6 at 10° or 15° C., but it did develop at 20° and 25° C.; at 30° C. it developed to a marked degree.

Differences in counter-clockwise growth at different temperatures were also noted in forms 4 and 7. In another series of cultures, form 9, when grown at 20° C., developed a very marked counter-clockwise growth of mycelium. In form 10, on the other hand, growing under identical conditions, the growth was clockwise. (See figure 5.)

Ustilago hordei. Consistent cultural differences were found in seven collections of *U. hordei.* Two forms were collected in the same field at University Farm, St. Paul, Minn., and two more were collected within a radius of one hundred miles from St. Paul. Others were obtained from widely separated localities. There can be no question regarding the distinctiveness of the different physiologic forms in culture. (See figure 6.) Detailed

TABLE 4.—*The influence of temperature on the counterclockwise growth of four physiologic forms of Ustilago nuda*

Form	Temperature in degrees C.				
	10	15	20	25	30
1	0[a]	+++	++	0	0
4	0	0	+	+	0
6	0	0	+	+	++
7	0	+	+	0	0

[a] 0 = absent
+ = present
++ = marked
+++ = very marked

descriptions of four of the seven forms identified are given in table 5. The range of variability of forms of *U. hordei* on culture media is very wide. The character and rate of growth and the ability to produce sporidia are influenced profoundly by environmental conditions. However, when grown under the same environmental conditions, the cultural characteristics are remarkably constant, as shown in figure 7.

Wedge-shaped sectors, which differed from the parents in color and rate of growth, and bred true in subsequent transfers, appeared in some of the cultures. Several explanations may be offered for their occurrence. Since

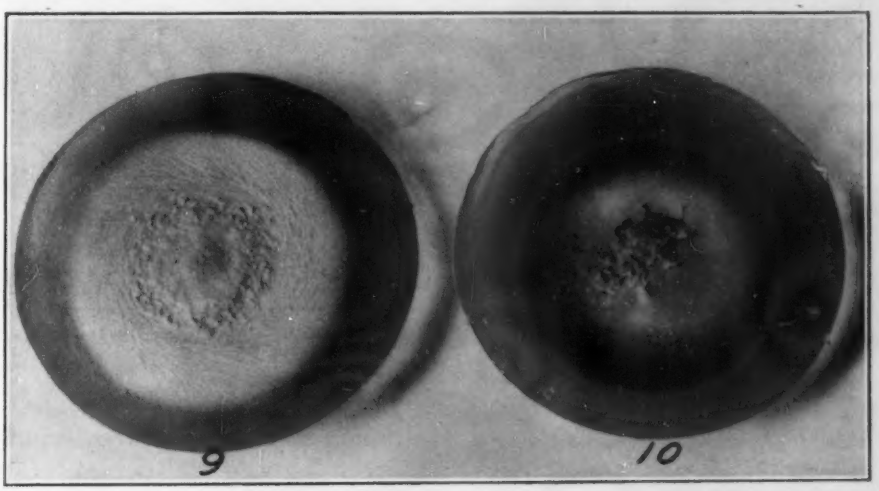

FIG. 5. Two physiologic forms of *Ustilago nuda* grown on potato dextrose agar at 20° C.: form 9, with counter-clockwise growth of mycelium; and form 10, with clockwise growth of mycelium.

the original isolations were not made from single chlamydospores but from a mass culture of a single collection, sectors possibly may have resulted from chance assortment of physiologic forms.

Kniep (28 and 29), Zillig (51), Bauch (2 and 3), Stakman and Christensen (45), and others have shown that there are sexual strains in the smut fungi. Zillig showed that there are sexual strains within the indi-

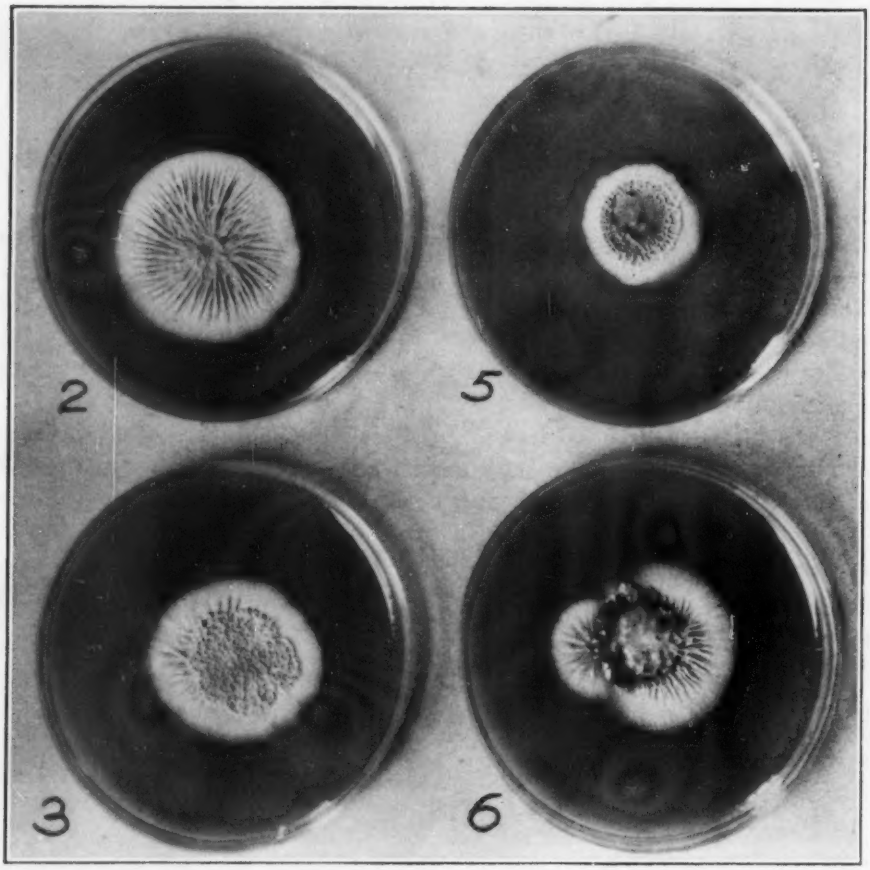

FIG. 6. Four physiologic forms of *Ustilago hordei* grown on potato dextrose agar: forms 2 and 3, from St. Paul, Minnesota; form 5, from Chaska, Minnesota; form 6, from Kansas.

vidual physiologic forms and also that fusions may occur between sexual strains of different physiologic forms. Stakman and Christensen (45) found that sexual strains of *U. zeae* differed in their appearance on culture media. Thus, the sectors might possibly have occurred as a result of segregation for sex. Christensen and Stakman (9) have shown mutation to be

TABLE 5.—*Cultural characteristics of physiologic forms of Ustilago hordei on potato dextrose agar, 24 days after inoculation*

Source of collection	Form	Color	Topography	Surface	Consistency	Margin
Italy E. Pantenelli	1	Vinaceous buff	Convex to pulvinate; center irregularly pitted and rough, felt-like; ray radially ridged	Waxy	Mycelioid and leathery	Undulate and mycelioid
St. Paul, Minnesota Writer	2	Avellaneous to wood brown	Convex; center irregularly pitted, tendency to become reticulate near the ray; ray with conspicuous counter-clockwise ridges	Waxy to dull	do	do
Chaska, Minnesota E. C. Stakman	5	Center wood-brown, changing to light buff in the ray portion	Center raised and coarsely reticulated; ray flat	do	Yeast-like and granular	Lacerate and mycelioid
Carney, Kansas H. E. Parson	6	Light pinkish cinnamon; sprinkling of white	Raised to convex; smooth	China-like	Bacterioid and slimy	do

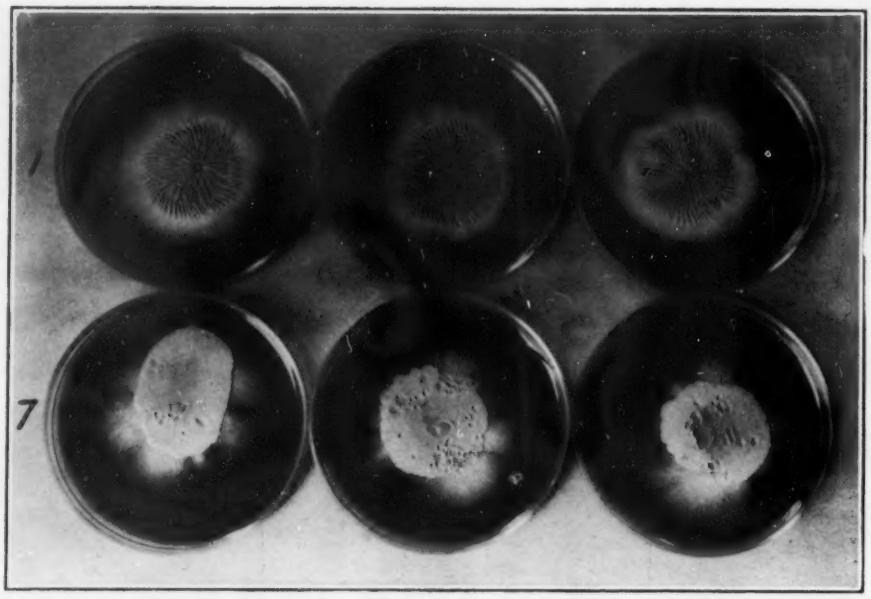

FIG. 7. Cultures showing uniformity of two physiologic forms of *Ustilago hordei* grown on potato dextrose agar: form 1, from Italy; form 7, from Iowa.

common in *U. zeae*. It is possible that these sectors in cultures of *U. hordei* also are the result of mutation. No sectoring such as occurred in *U. hordei* has ever been seen by the writer in cultures of *U. tritici* or *U. nuda*, although these smuts were grown for several years on several different kinds of media and under different environmental conditions.

FIG. 8. Three physiologic forms of *U. levis* grown on potato dextrose agar: form 1, from Minnesota; form 3, from Virginia; form 5, from China.

Ustilago levis. Five distinct physiologic forms of *U. levis* were differentiated on the basis of cultural characteristics. Detailed descriptions of three of the forms are given in table 6. There were decided differences in cultural characteristics of these forms (Fig. 8)—in fact greater in some cases than were the differences between cultures of *U. levis* and *U. avenae.* It should be emphasized again that the cultural differences between the forms can be detected only when the proper differential materials are used. Furthermore, there are great differences between forms grown under one particular set of environmental conditions, which are not apparent when the forms are grown in another environment. No consistent microscopic differences were noted in the vegetative growth of *U. levis* and *U. avenae.* No sectors appeared in the cultures of any of the five forms of *U. levis.*

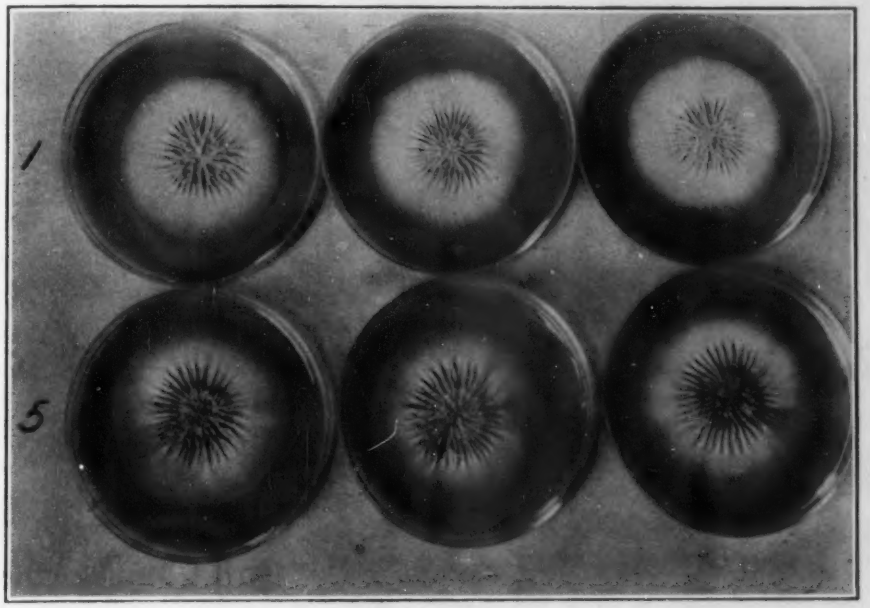

FIG. 9. Cultures showing uniformity of two physiologic forms of *Ustilago levis* grown on potato dextrose agar: form 1, from Minnesota; form 5, from China.

Ustilago avenae. From 22 collections of *U. avenae* that were obtained, 17 physiologic forms were differentiated on the basis of cultural characteristics. Another form was obtained as a result of sectoring in the form originally collected in South Dakota. (See table 1 for the origin of the various forms.) The data on cultural characters of six forms are summarized in table 7. Here again there were decided differences in the cultural characteristics. (See figure 10.)

TABLE 6.—*Cultural characteristics of physiologic forms of Ustilago levis on potato dextrose agar, 24 days after inoculation*

Source of collection	Form	Color	Topography	Surface	Consistency	Margin
St. Paul, Minnesota Writer	1	Smoke gray	Convex; prominent radial ridges of unequal length	Waxy	Mycelioid and leathery	Entire and mycelioid
Virginia R. M. Nelson	3	Pale olive buff to pearl gray	Convex; smooth to warty	Wet, shiny	Bacterioid and slightly mycelioid	Entire, bacterioid to mycelioid
China C. C. Chen	5	Cream interspersed with mummy-brown	Convex to umbonate; smooth to coarsely ridged; center slightly tufted; slight counter-clockwise growth	Waxy	Mycelioid and leathery	Entire and mycelioid

TABLE 7.—*Cultural characteristics of physiologic forms of Ustilago avenae on potato dextrose agar, 24 days after inoculation*

Source of collection	Form	Color	Topography	Surface	Consistency	Margin
St. Paul, Minnesota Writer	1	Pale olive buff	Raised; mycelioid center 20 mm. in diameter, radially ridged, becoming smooth in ray portion	Wet, shiny	Center mycelioid; ray bacterioid	Lobate; beaded to sparsely mycelioid
Germany T. E. Roemer	2	Buff olive to drab; margin pale gray	Convex to umbonate; numerous daedaloid counter-clockwise ridges; margin of short shallow radial ridges	Waxy	Mycelioid and leathery	Appressed, entire and mycelioid
Arcadia, Wisconsin J. J. Christensen	16	Prout's brown; margin of 5 mm. pearl gray	Convex to umbonate; numerous chain-like radial ridges, irregular in size and length	Moist to waxy	do	do
Chickasha, Oklahoma W. Butler	5	Mummy brown center 20 mm. diameter, ivory in ray portion; margin pearl gray to yellow	Slightly umbilicate with high compact radial ridges	Center glossy; ray waxy	Center chiefly bacterioid; remainder mycelioid and leathery	Mycelioid and plumose
Dallas, Texas W. Butler	9	Light buff to pale gull gray	Umbonate; center of upright tufts; numerous shallow irregular fine chain-like ridges	Wet, shiny	Mycelioid and leathery	Slightly wavy and mycelioid
Sector from form 12	13	Center of 8 mm. light pinkish cinnamon; ray portion mummy-brown; margin pearl gray	Convex; center crater-like, sparsely tufted; ray portion of regular narrow ridges	do	do	Appressed, mycelioid, plumose

With one exception, the type of growth and ability to produce sporidia could be influenced easily by changing the environmental conditions. In the original transfer, form 2 produced sporidia abundantly. However, in as many as 25 subsequent transfers to several different kinds of media, containing different amounts of moisture and stored at different temperatures, cultures of this particular form consisted almost entirely of mycelium. Forms 11 and 12 produce sporidia more abundantly than do any of the other forms, but they can always be made to produce some mycelium by decreasing the amount of moisture in the culture media.

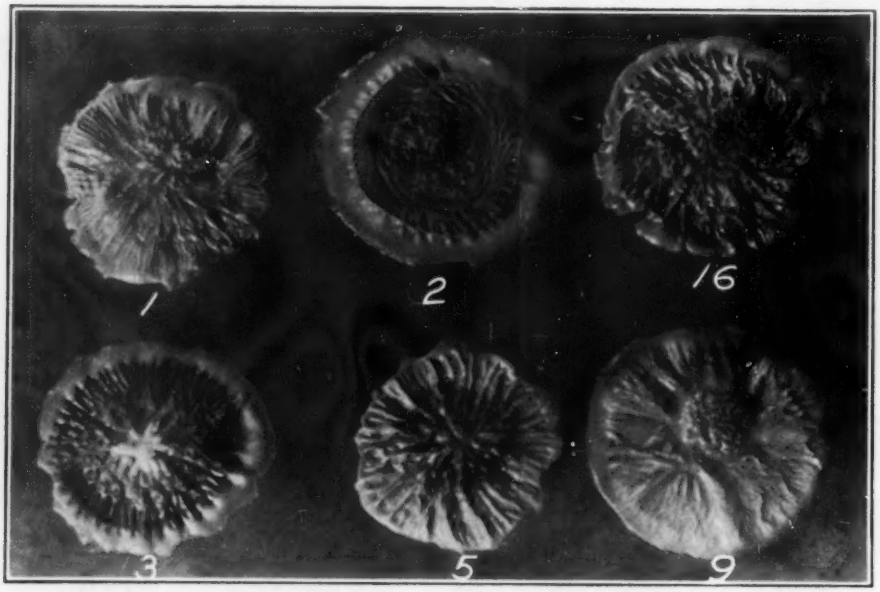

FIG. 10. Six physiologic forms of *Ustilago avenae* grown on potato dextrose agar: form 1, from Minnesota; form 2, from Germany; form 16, from Wisconsin; form 13, from sector of form 12; form 5, from Oklahoma; form 9, from Texas.

EFFECT OF TEMPERATURE ON THE GROWTH OF PHYSIOLOGIC FORMS

Differences in the way in which cultures of physiologic forms of various fungi react to temperature have been noted previously. Edgerton (13) was able to distinguish between two physiologic forms of *Glomerella cingulata* (Atk.) S. and S., by means of temperature relations.

Similar differences were shown between strains of *Rhizopus nigricans* Ehrnb., by Harter and Weimer (21). One strain of *R. nigricans* was found to have a lower optimum than seventeen others. Johnson (27) found different temperature requirements for two physiologic forms of *Helmintho-*

sporium gramineum Rab. No appreciable differences of growth occurred at the optimum temperature, which was the same for both forms, but one grew decidedly better than the other at low temperature (5–6° C.). No such differences have been shown between cultures of physiologic forms of the smut fungi.

In order to determine whether the physiologic forms of some of the smuts could be distinguished on the basis of reaction to temperature, a series of tests was made as follows: To each of a triplicate series of 200 cc. Erlenmeyer flasks were added 35 cc. of the same preparation of 2 per cent potato dextrose agar. The flasks were then inoculated with small, but approximately equal, portions of each culture and all incubated at room temperature for a period of 24 hours. Thus the different forms were subjected as nearly as possible to identical environmental conditions before distribution to various temperatures. All of the forms were incubated simultaneously at each of the following temperatures: 10°, 15°, 20°, 30°, and 35° C. The diameter of each colony was measured at the end of 14 days and again at the end of 21 days. Differences in rate of growth were apparent at the end of 14 days but were more striking at the end of 21 days. The figures given are therefore based on the diameters of the colonies at the end of 21 days. As there was very little difference in the size of colonies in replicated cultures of some forms, only the averages are recorded in the tables. In order to ascertain whether the physiologic forms would react the same to different temperatures after a period of time, the experiments made with *U. tritici* and *U. nuda* in 1927 were repeated one year later. During this time transfers had been made to fresh media six different times and the cultures subsequently were stored under the same environmental conditions.

Ustilago tritici. The results of the experiment with four physiologic forms of *U. tritici* are summarized in table 8 and the data obtained in 1927 are graphically represented in figure 11. It seems clear from table 8 and figure 11 that there are differences in the reactions of some physiologic forms of *U. tritici* to temperature. There were differences in the optimum temperature for growth, as indicated by the size of colonies and also in the total amount of radial growth at certain temperatures. (See figure 12.) The optimum temperature for form 4 was 20° C. For forms 1, 2, and 5, it was 25° C. Form 2 is distinct from forms 1, 4, and 5 in that it has a wider temperature range and also the amount of radial growth attained by it is greater at all temperatures. In 1927, at the end of 21 days incubation at 35° C., the average diameter of the colonies of form 2 was 6.7 mm., while cultures of forms 1, 4, and 5 failed to grow at all. There was a difference of 7 mm. in the diameter of growth between forms 1 and 2 at 10° C. The differences in the amount of radial growth are not so marked between forms

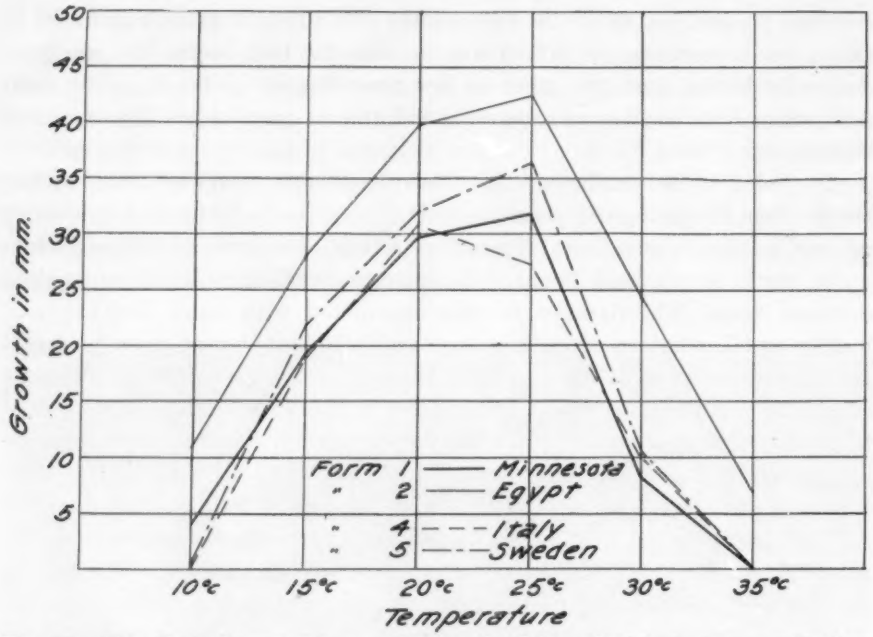

FIG. 11.—The effect of temperature on the growth of four physiologic forms of *Ustilago tritici* grown on potato dextrose agar for 21 days, in 1927.

1 and 5 as between some of the other forms. At 20° C. there is a difference of only 3.3 mm. in the diameter of the colonies, while at 25° C., the optimum

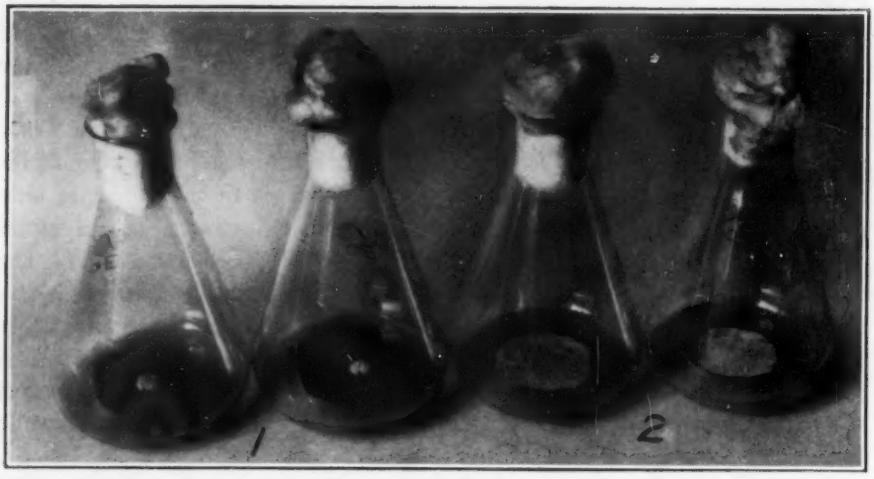

FIG. 12.—Two physiologic forms of *Ustilago tritici* grown on potato dextrose agar at 30° C.: form 1, from Minnesota; form 2, from Egypt.

TABLE 8.—*Influence of temperature on the growth of four physiologic forms of Ustilago tritici grown on potato dextrose agar for 21 days*

Form number	Temperature in degrees C. and diameter of colonies in mm.																	
	10			15			20			25			30			35		
	Series[a]			Series			Series			Series			Series			Series		
	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.
1	4	3.3	3.7	19.3	16.3	17.8	29.7	27.3	28.5	31.7	30.0	30.9	7.7	6.7	7.2	0.0	0.0	0.0
2	11	9.0	9.5	28.0	19.3	23.7	39.7	35.0	37.4	42.3	39.0	40.7	24.3	23.0	23.7	6.7	2.0	4.4
4	0.0	Tr.	Tr.	19.0	17.7	18.4	30.7	27.3	29.0	27.3	21.3	24.3	9.7	8.0	8.9	0.0	0.0	0.0
5	Tr.	Tr.	Tr.	21.7	16.0	18.9	32.0	25.7	28.9	36.3	24.0	30.2	10.3	7.7	9.0	0.0	0.0	0.0

[a] Series 1 run in October, 1927; Series 2 in October, 1928.

temperature for their growth, this difference is slightly increased to 4.6 mm. Form 4 is distinct from forms 1 and 5 in that it has a different optimum temperature for growth.

The experiments which were made with the various physiologic forms of *U. tritici* in October, 1927, were repeated in October, 1928. During this period the cultures were transferred several times at 60-day intervals to fresh media, once to 2 per cent malt-extract agar and five times to 2 per cent potato dextrose agar. When again tested in 1928 for differences in their reaction to temperature, they behaved in the same manner as they had in 1927. The diameter of the colonies in 1928 was slightly less in all cases than that in 1927, but this may have been due to a very slight difference in the media used in the two years.

Ustilago nuda. Five physiologic forms of *U. nuda* were tested for differences in their growth reactions at the various temperatures noted above. The data obtained are summarized in table 9, and the 1927 results are represented graphically in figure 13. It is evident that there are differences in the reaction to temperature of physiologic forms of *U. nuda.* (See figures 14 and 15.) There were differences in the optimum temperature for growth of certain physiologic forms and differences in the amount of radial growth at certain temperatures. The optimum temperature for

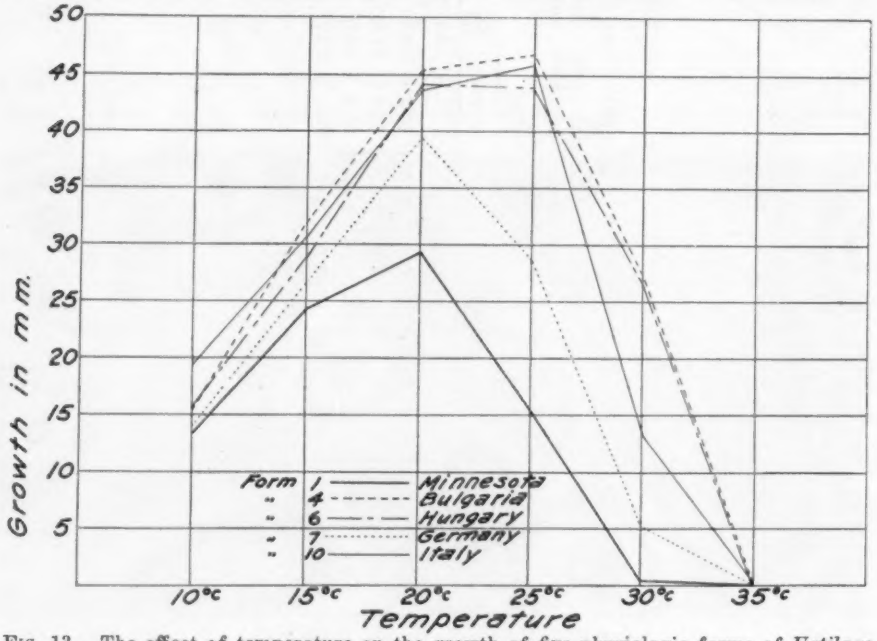

FIG. 13. The effect of temperature on the growth of five physiologic forms of *Ustilago nuda* grown on potato dextrose agar for 21 days, in 1927.

TABLE 9.—*The influence of temperature on the growth of five physiologic forms of Ustilago nuda grown on potato dextrose agar for 21 days*

Form number	Temperature in degrees C. and diameter of colonies in mm.																	
	10			15			20			25			30			35		
	Series[a]			Series			Series			Series			Series			Series		
	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.
1	13.3	8.7	11.0	24.3	17.3	20.8	29.2	22.3	25.8	15.3	16.0	15.7	Tr.	0.0	Tr.	0.0	0.0	0.0
4	15.5	12.3	13.9	32.0	24.3	28.2	45.5	40.7	43.1	46.7	45.7	46.2	27.0	23.0	25.0	Tr.	0.0	Tr.
6	15.7	12.7	14.2	28.8	24.0	26.4	44.2	35.0	39.6	44.0	32.3	38.2	26.7	26.3	26.5	0.0	0.0	0.0
7	13.7	.....	13.7	26.8	.....	26.8	39.5	.....	39.5	28.7	.....	28.7	5.2	.....	5.2	0.0	.....	0.0
10	16.3	15.0	15.7	30.7	31.0	30.9	43.7	41.7	42.7	45.8	46.7	46.3	13.2	20.3	16.8	Tr.	0.0	Tr.

[a] Series 1 run in October, 1927; Series 2 in October, 1928.

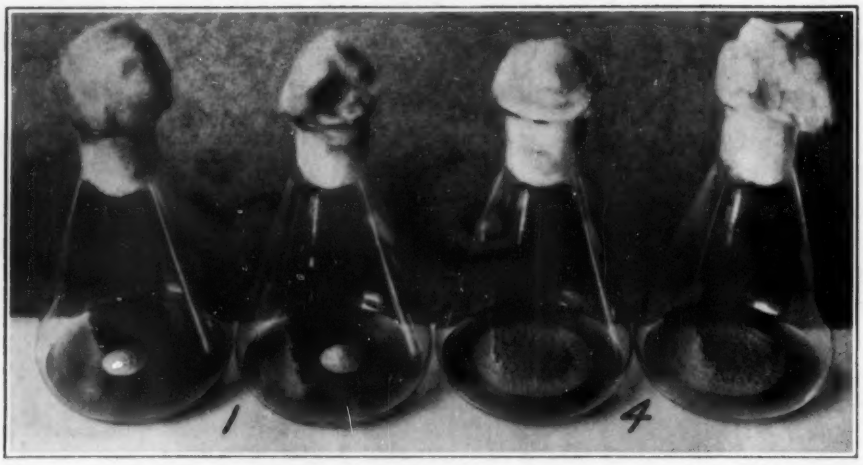

FIG. 14. Two physiologic forms of *Ustilago nuda* grown on potato dextrose agar at 25° C.: form 1, from Minnesota; form 4, from Bulgaria.

growth of forms 1 and 7 was 20° C. and that of forms 4 and 10, 25° C. Although forms 1 and 7 have the same optimum temperature for growth, they are distinctly different as regards the amount of radial growth at certain temperatures. In the 1927 tests these two forms grew about equally well at 10° C. At 20° C., however, the culture of form 7 grew, on the average, 10.3 mm. in diameter more than did form 1. Likewise, at 25° C. the difference in diameter of the colonies of these two forms was 13.4 mm.

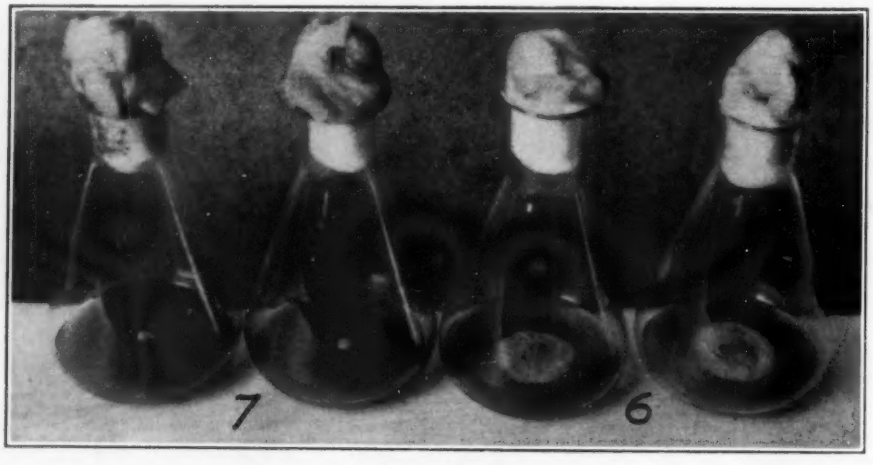

FIG. 15. Two physiologic forms of *Ustilago nuda* grown on potato dextrose agar at 30° C.: form 7, from Germany; form 6, from Hungary.

At 30° C. the differences were less marked, and at 35° C. neither form grew at all. The differences in the amount of radial growth made by forms 4, 6, and 10 were very slight and evidently not significant. As a group, however, they reacted distinctly differently from forms 7 and 1. At 10° and 15° C. the differences in the amount of growth of all the forms was not great, but at 25° C. the differences between forms 4, 6, and 10 as a group, and forms 7 and 1, were very wide. The average diameter of the colonies of form 1 at this temperature was 15.3 mm.; form 7, 28.7 mm.; and of forms 4, 6, and 10, it was 46.7, 44.0, and 45.8 mm. respectively.

The cultures of the physiologic forms of *U. nuda* tested in October, 1927, for differences in their reaction to temperature were again tested in October, 1928. They were transferred to fresh media and stored as described for similar tests with physiologic forms of *U. tritici*. Here again the results obtained in 1928 agreed with those obtained the previous year. The cultural characteristics of any one physiologic form of the smuts tested were found to be very constant when the cultures were grown at the same temperature. By changing the temperature, however, variations were induced, particularly in the topography of the colonies.

Ustilago hordei. It seems clear from table 10 and figure 16 that physiologic forms of *U. hordei* react differently when grown at certain tempera-

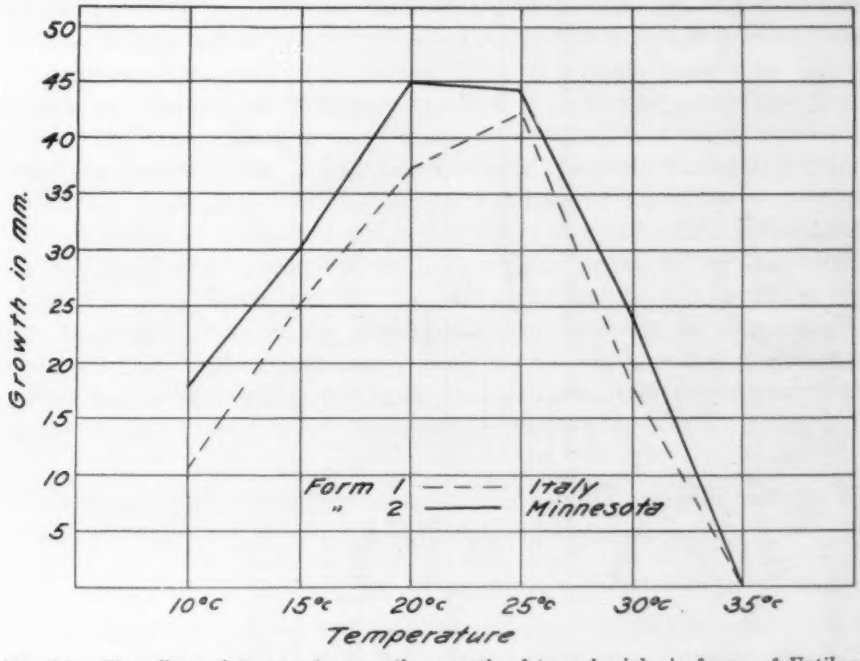

FIG. 16. The effect of temperature on the growth of two physiologic forms of *Ustilago hordei* grown on potato dextrose agar for 21 days, in 1928.

TABLE 10.—*The influence of temperature on the growth of two physiologic forms of Ustilago hordei grown on potato dextrose agar for 21 days*

Form number	Temperature in degrees C. and diameter of colonies in mm.																	
	10			15			20			25			30			35		
	Series			Series			Series			Series			Series			Series		
	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.
1	9.0	10.0	10.5	23.0	27.7	25.4	35.7	38.7	37.2	42.0	42.5	42.3	17.7	18.0	17.9	0.0	0.0	0.0
2	15.7	18.0	16.9	30.0	31.3	30.2	44.3	45.5	44.9	42.7	46.0	44.4	23.0	25.3	24.2	0.0	0.0	0.0

tures, as did the forms of *U. tritici* and *U. nuda*. Only two forms were used in the test, but it is very probable that, had this number been increased, more differences would have been found.

Form 1 has an optimum temperature somewhere between 20° and 25° C., while that of form 2 is 25° C. At all temperatures between 10° and 30° C. the diameter of the colonies of form 2 was greater than that of form 1. At 25° C., however, the difference in the diameter was only 2.1 mm. while at 20° C. this difference was widened to 7.7 mm. At 35° C. cultures of neither form developed. Series 1 of this experiment was run in November, 1927. Two months later the experiment was repeated. In the meantime the cultures of the two physiologic forms had been transferred at intervals of two weeks to a 2 per cent malt extract, then to 2 per cent malt agar, and finally to a 2 per cent potato dextrose. The reaction of the physiologic forms to temperature in the second series was similar to that in the first series.

Ustilago levis and *Ustilago avenae*. Three physiologic forms of *U. levis* and four of *U. avenae* were tested for differences in reaction to temperature. The differences in the amount of radial growth of physiologic forms of both species were very slight and evidently fall within the limits of experimental error.

Physiologic forms of some of the smut fungi can thus be differentiated by differences in the optimum temperature for growth and in the amount of growth attained at certain temperatures. This is not only interesting but may be of practical importance. The fact that there are these differences may account for the development of a particular physiologic form in a certain geographical area. Furthermore, it may account for variation from year to year in the susceptibility to smut of certain varieties of grain.

Fromme (17) reported that the percentages of wheat heads affected with *U. tritici* varied according to the degree of soil fertility maintained. He concluded that it seems probable that these variations are due to the total or partial elimination of the fungus by the greater vigor of the growth of the plants on the more fertile soil. It would be interesting to know, then, what the results would be if the temperature factor were controlled and a vigorously growing physiologic form of *U. tritici* were used, as for example form 2, which was collected in Egypt.

PATHOGENIC DIFFERENCES BETWEEN PHYSIOLOGIC FORMS AND VARIETAL RESISTANCE OF WHEAT, BARLEY, AND OATS TO SMUT

Varietal resistance of spring wheat to Tilletia levis

In connection with the study of the pathogenicity of physiologic forms of bunt, a study was made of the varietal resistance of spring wheat to

Tilletia levis Kühn. Heretofore much of the work on bunt resistance has been done with winter wheat varieties. Woolman and Humphrey (50) summarized the literature up to 1924 on varietal resistance to bunt. Heald and Woolman (22), Stephens and Woolman (48), Coons (10), Johnston (25 and 26), and Gaines (18 and 19) have also recently reported on varietal resistance to bunt.

In 1919, about 870 varieties and strains of spring wheats were tested for varietal resistance to *T. levis* in Minnesota. This number was reduced from year to year until by 1923 all except about 40 of the most important varieties had been dropped. The results were reported by Stakman, Lambert, and Flor (46). They concluded that the durums, as a class, were more resistant than the common wheats, but that there were wide differences within both classes.

The varieties used by Stakman *et al.* (46), in 1923, were tested for varietal susceptibility by the writer from 1924 to 1927 inclusive. The seed was very heavily smutted artificially a few days before sowing, and each variety was grown in triplicate rod rows systematically distributed throughout the plot. Shortly after harvest, 200 heads were selected at random from the rod row bundles of each variety. The percentage of bunted heads was then determined from this number.

The results presented in table 11 indicate that there is considerable variation in the amount of bunt developed within the individual groups of wheat. There appears, nevertheless, to be a general correlation between the species group and the degree of resistance. Eighteen varieties and selections of *Triticum vulgare* were grown, and the average percentage of bunted heads was 27.1. In eighteen varieties and selections of durum the average was 7.6 per cent; for two varieties of emmer, 12.3 per cent; and for one variety of einkorn there was an average of 23.6 per cent of bunt. Einkorn, however, is much more resistant than this percentage seems to indicate, for in practically every case the heads were only partially smutted.

There was considerable difference in the amount of bunt in the varieties of common wheat. Marquis, C. I. 3641, with 7.9 per cent of bunt, was the most resistant during the period of four years. Parker, Minn. 2222, Marquillo, C. I. 6887, and Quality, C. I. 6607, had 8.7, 9.5, and 9.7 per cent of bunt respectively and are apparently in the same class with Marquis. Ceres, C. I. 6900, appeared most susceptible, with 52.8 per cent of bunt. Kota, C. I. 5878, and Progress, C. I. 6902, were practically the same with 52.6 and 50.7 per cent respectively.

Of the durum wheats, Kubanka, C. I. 2094, with 2.4 per cent of bunt, and Akrona, C. I. 6881, with 3.2 per cent, were the most resistant varieties. Arnautka, C. I. 1431, with 15.5 per cent of bunt, and Acme, C. I. 5284, with

13.2 per cent, were most susceptible. Many of the heads of the durum varieties were only partially smutted.

The fact that there are resistant varieties in each group of wheat, that is, in the 42-, the 28-, and 14-chromosome groups, indicates that resistance is not correlated with any particular morphologic type. It should then be possible to breed varieties of wheat which are desirable agronomically and which are highly resistant to bunt. Gaines (19) already has called attention to these facts and has developed at least one good wheat, Ridit, which for a time was immune from *Tilletia tritici* in the Palouse district of the West. The results of the tests recorded in table 11 indicate that the production of bunt-resistant, hard-red common wheats should not be difficult. The question arises, however, as to whether those varieties which are resistant to bunt in one locality will be resistant to bunt which occurs in other geographical areas. In general the percentages of bunt obtained by the writer were much higher than those obtained by Stakman, Lambert, and Flor (46). In tests made each year from 1919 to 1923 these investigators obtained an average of only 0.7 per cent of bunt in Marquis, C. I. 3641. Using the same variety each year from 1924 to 1927, the writer obtained an average of 7.9 per cent. In similar comparisons the average percentage of bunt in Kota, C. I. 5878, was increased from 16.5 to 52.6; in Pentad, C. I. 3822, from 0.3 to 8.9; and in Arnautka, C. I. 1431, from 2.4 to 15.5. Einkorn, C. I. 2433, when tested by Stakman *et al* was found to be immune to bunt, but in the writer's tests an average of 23.6 per cent developed.

Increases in the percentage of bunt of wheat in 1924 were not confined to varieties grown at University Farm, St. Paul, Minnesota. During the summer of 1924 there was an unusually high percentage of bunt in Marquis and in durum wheats throughout the entire hard red spring wheat region. The following summer there was an unusual outbreak of bunt, caused, in Minnesota, at least, principally by *T. levis*, although *T. tritici* was found to some extent. The outbreak might be accounted for in two ways: either the weather and soil conditions were unusually favorable for the development of bunt or there may have been an unusually virulent strain of the pathogene. The latter explanation seems the more probable because bunt has continued to be epidemic in the durums since the first outbreak. Furthermore, Faris (15) obtained some preliminary evidence of differences in pathogenicity of *T. levis* and *T. tritici*. Work was therefore undertaken to ascertain whether there actually are distinct physiologic forms of *T. levis* and *T. tritici*. A preliminary paper was published by Rodenhiser and Stakman (40) on the results obtained in 1926. The work was extended in 1927 and the data for both years are given in this paper.

TABLE 11.—*The percentage of bunt (Tilletia levis) on wheat varieties at University Farm, St. Paul, Minnesota, in 1924–1927*

Species and varieties	C. I. Number	Year and percentage of smutted heads				
		1924	1925	1926	1927	Average
Triticum vulgare						
Ceres	6900	—	63.5	41.0	54.0	52.8
Glyndon Fife	2873	54.0	44.8	29.2	45.7	43.4
Hard Federation	4733	34.0	16.2	17.2	40.3	26.9
Haynes Bluestem	2874	39.0	23.0	15.8	12.0	22.5
Kitchener	4800	28.0	36.6	20.6	37.2	30.6
Kota	5878	52.0	56.2	35.7	66.3	52.6
Marquillo	6887	15.1	6.5	6.0	10.5	9.5
Marquis	3641	9.0	8.2	3.2	11.0	7.9
Marquis (Parker's) Minn. 2222	——	—	3.5	12.0	10.5	8.7
Power	3697	—	13.0	21.5	3.0	12.5
Prelude	4323	43.0	23.7	39.7	61.2	41.9
Preston	2958	26.0	7.5	12.0	16.2	15.4
Preston	3021	22.0	30.2	20.8	26.3	24.8
Progress	6902	—	49.5	30.5	72.0	50.7
Quality	6607	—	14.5	4.0	10.5	9.7
Red Bobs	2157	47.0	27.6	22.2	40.0	34.2
Ruby	2135	31.0	17.2	10.3	29.3	22.0
Stanley Fife	1594	18.0	28.8	10.5	32.3	22.4
Average						27.1
Triticum compactum						
Washington little club	4066	—	—	—	67.5	67.5
Triticum durum						
Acme	5248	13.0	6.5	8.2	25.0	13.2
Akrona	6881	—	4.0	1.0	4.5	3.2
Arnautka	1431	18.0	15.8	7.0	21.3	15.5
Arnautka	1494	11.0	3.8	1.0	11.2	6.8
Arnautka	1537	5.0	5.5	0.2	10.0	5.2
Bolley's D 7	3323	14.0	3.7	6.2	15.8	9.9
Iumillo	1736	7.0	1.5	0.3	10.5	4.8
Kahla	2088	8.0	1.3	5.0	6.0	5.1
Kubanka	1354	4.0	3.0	1.7	—	2.9
Kubanka	1516	11.0	9.5	3.2	28.3	13.0
Kubanka	1440	—	11.3	5.7	22.2	13.1
Kubanka	2094	3.0	0.7	1.2	4.5	2.4
Kubanka	2234	4.0	2.8	1.0	10.3	4.5
Kubanka	2952	3.0	3.7	0.3	9.0	4.0
Mindum	5296	6.0	1.3	0.5	18.5	6.6
Nodak	6519	—	3.5	7.0	11.0	7.2
Monad	3320	14.0	4.2	2.6	19.0	10.0
Pentad	3822	13.0	3.0	5.7	13.7	8.9
Average						7.6
Triticum dicoccum						
Khapli	4013	7.0	2.1	5.5	18.7	8.3
White Spring Emmer	1524	11.0	13.8	0.5	38.7	16.0
Average						12.3
Triticum monoccum						
Einkorn	2433	19.0	33.8	5.8	35.7	23.6

Pathogenicity of physiologic forms of Tilletia levis and Tilletia tritici

Five collections of *T. levis* and seven of *T. tritici* were obtained from the places indicated in tables 12 and 14.

In the spring of 1925 Kota wheat, C. I. 5878, was inoculated with each collection of smut, except those from California and Washington which had not yet been obtained. The smutted heads which resulted were then picked and kept under the same conditions until the spring of 1926. Likewise, the inoculum used in the spring of 1927 was that which had developed on Kota wheat the previous year and was then stored under the same conditions until used in 1927. Thus the spores of all of the collections, with the exception mentioned, were produced and kept under similar conditions.

Mindum, C. I. 5296, a durum wheat; Einkorn, C. I. 2433; Marquis, C. I. 3641, and Kota, C. I. 5878, both hard red spring wheats, were inoculated with chlamydospores of each collection of smut. In 1927, Pentad, C. I. 3822, a durum wheat, was substituted for the Mindum used in 1926. Previous to inoculation, the seed was treated by Jensen's modified hot water method. When thoroughly dry, the seed was inoculated with powdered inoculum from each collection, at the rate of 0.5 grams to 100 grams of seed. All the seed was then sown on the same day, in 1926, in duplicate and in 1927 in triplicate systematically distributed rod rows. As there was very little difference in the percentage of smut in the replicated rows, only the averages are recorded.

With one exception, the percentages of infection obtained in 1926, as given in tables 12 and 14, are based on counts of 800 heads of Einkorn, 800 of Marquis, and 1000 of Kota. The counts on the varieties inoculated with the Egyptian collection are based on counts of 400 heads of Einkorn, 400 of Marquis, and 500 of Kota. Each individual head of wheat was cut in order to detect partial smutting. The percentages of partially and completely smutted heads of Einkorn and Marquis were recorded separately. In 1926 the number of partially smutted heads of Kota was not determined, because there was very little partial smutting. So little smut developed in Mindum that the results are not recorded. The percentages of infection obtained in 1927, recorded in tables 13 and 15, are based on counts of 900 heads of each variety. Three hundred heads were selected at random from each of the triplicated rod rows, but here again the differences in the percentage of smut in the replicated rows was so small that averages only are recorded.

The results of the two-year tests are recorded in tables 12 to 15 inclusive and the data obtained in 1926 are presented graphically in figures 17 and 18. The varieties of wheat used in the tests have been grown at University Farm, St. Paul, Minn., for eight years, from artificially smutted seed, and

with one exception there has always been a high degree of correlation between the percentage of bunt in the different years, as well as in the replicated rod rows. During the last four years, however, a much higher percentage of bunt has developed in practically all of the varieties than in the previous seasons.

It is evident from figures 17 and 18 and tables 12, 13, 14, and 15, that there are sufficiently great differences in the virulence of several collections of smut to justify the conclusion that there are physiologic forms which differ in pathogenicity. In table 16 is indicated the relative susceptibility of each variety to each of these forms in 1926. It seems clear from these tables that there are at least three physiologic forms of *T. levis*. The two collections from Hungary were so nearly alike in 1926 that they are considered to be identical and are designated as form 1. In comparison with the behavior of form 1, in 1926, the collection from Minnesota was slightly more virulent on Einkorn, about the same on Marquis, but was less virulent on Kota.

In 1927 similar results were obtained, with the exception that the Minnesota collection was somewhat less virulent than form 1 on Marquis. In an additional test with Pentad, in 1927, this collection proved to be less virulent than form 1. It is considered as form 2. The collection from Egypt, when tested in 1926, was more virulent on Einkorn than the other two, decidedly less virulent on Marquis, and somewhat less virulent on

TABLE 12.—*The percentage of smutted heads in Marquis and Kota wheats and in Einkorn inoculated artificially with five collections of Tilletia levis at University Farm, St. Paul, Minn., in 1926*

Source of inoculum	Germination of chlamydospores in per cent	Percentage of smutted heads						
		Einkorn			Marquis			Kota
		Partial	Complete	Total	Partial	Complete	Total	Total
Minnesota	65	8.4	6.7	15.1	0.4	7.1	7.5	64.3
Hungary[a]	80	12.7	1.9	14.6	0.9	4.6	5.5	61.7
Hungary[b]	75	7.4[c]	1.5	8.9	1.7	5.3	7.0	61.1
Italy	80	13.4	7.3	20.7	1.2[c]	0.2	1.4	56.7
Egypt	90	9.8	8.3	18.1	0.3[c]	0.3	0.6	37.6
Uninoculated	—	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a] Hatvan

[b] Debreizen

[c] Individual heads lightly smutted. Only a few smutted, or partially smutted kernels in each hand.

TABLE 13.—*The percentage of smutted heads in Marquis, Kota, and Pentad wheats and in Einkorn inoculated artificially with four collections of Tilletia levis at University Farm, St. Paul, Minn., in 1927*

Source of inoculum	Germination of chlamydospores in per cent	Percentage of smutted heads											
		Einkorn			Marquis			Kota			Pentad		
		Partial	Complete	Total	Partial	Complete	Total	Partial	Complete	Total	Partial	Complete	Total
Minnesota	85	30.5	1.0	31.5	3.4	1.9	5.3	5.0	14.5	19.5	2.9	0.4	3.3
Hungary[a]	80	22.4	1.6	24.0	7.2	4.0	11.2	6.8	18.3	25.1	4.6	0.9	5.5
Italy	60	41.8	5.9	47.7	1.9	0.5	2.4	9.5	18.4	27.9	4.3	1.2	5.5
Egypt	70	46.6	8.0	54.6	0.3	0.1	0.4	11.2	24.0	35.2	10.9	4.1	15.0
Uninoculated	—	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a] Debreizen.

Kota. In 1927 similar results were obtained except that this collection was more virulent on Kota. When tested on Pentad in 1927, it was found to be decidedly more virulent than either form 1 or 2. This collection therefore is designated as form 3. The collection from Italy is somewhat similar to the one from Egypt, although it is much less virulent on Pentad. This may be another form, but until further tests are made it is not so considered.

One might conclude that there are several different forms of *T. tritici* (tables 14 and 15). The New Zealand collection was consistently less virulent in both the 1926 and 1927 tests than that from Norway; and the differences are so great that one is forced to the conclusion that they represent two distinct forms. The collection from New Zealand is therefore desig-

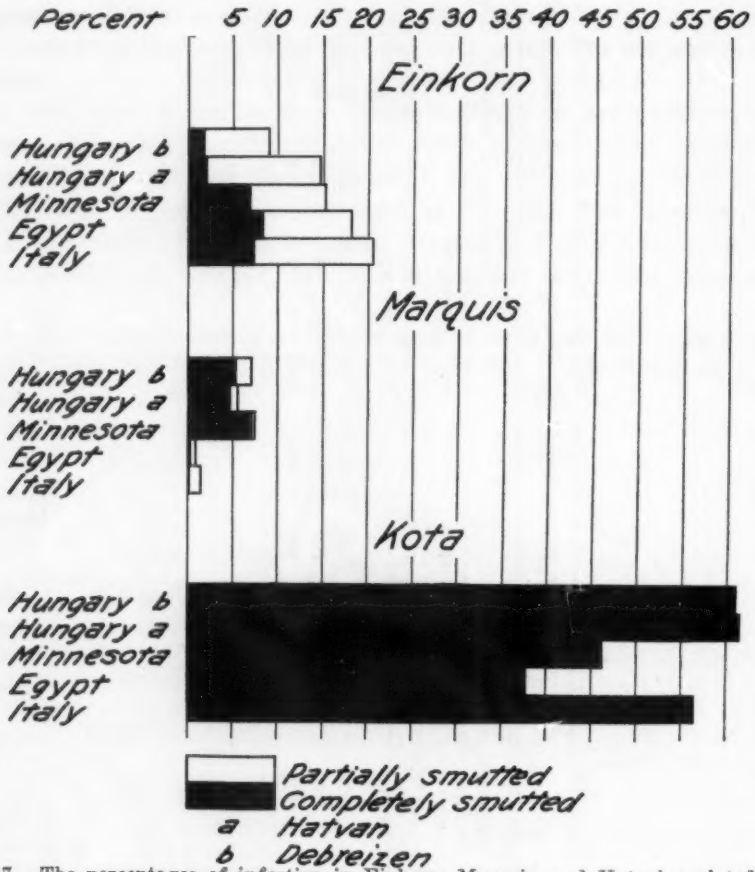

FIG. 17. The percentages of infection in Einkorn, Marquis, and Kota inoculated with five collections of *Tilletia levis*, in 1926.

nated as form 1 and the one from Norway as form 2. For the present the collection from Hungary is considered to be form 2. The collection from Washington is similar to form 1 on Marquis, Kota, and Pentad but is more virulent on Einkorn. It probably is a third form, but until this collection, as well as those from Sweden, Manitoba, and California, are tested on other varieties of wheat, no definite form numbers are assigned to them.

The results of these tests indicate that, although one may breed varieties of wheat which are highly resistant to bunt in one locality, there is no as-

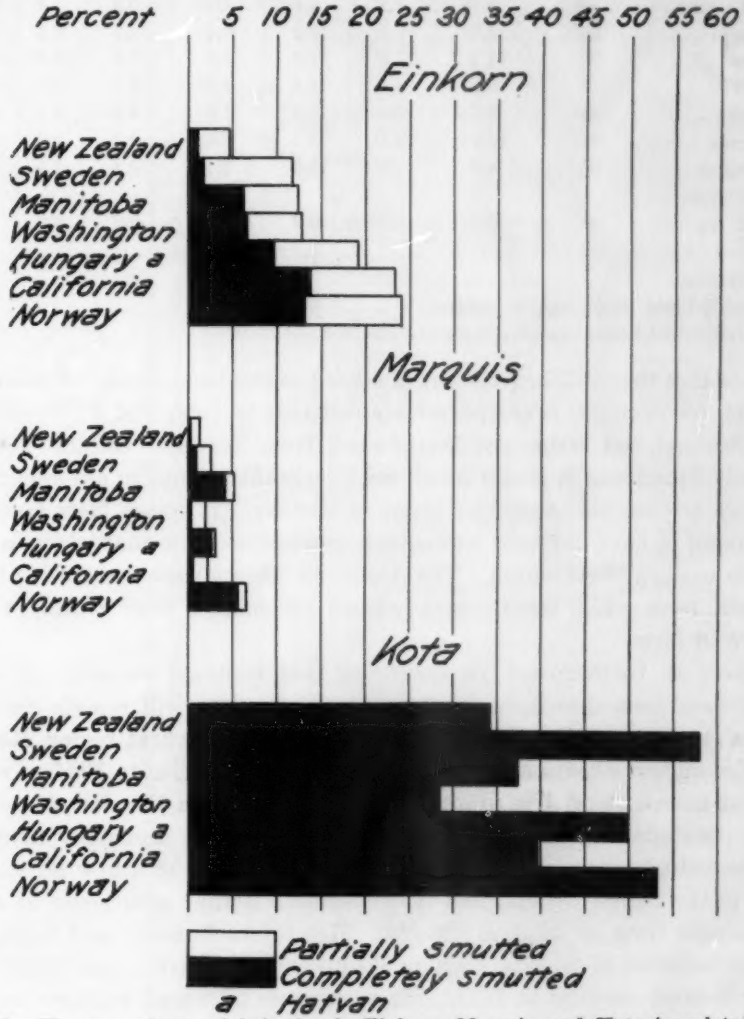

FIG. 18. The percentages of infection in Einkorn, Marquis, and Kota inoculated with seven collections of *Tilletia tritici* in 1926.

TABLE 14.—*The percentage of smutted heads in Marquis and Kota wheats and in Einkorn inoculated with seven collections of Tilletia tritici at University Farm, St. Paul, Minn., in 1926*

Source of inoculum	Germination of chlamydospores in per cent	Percentage of smutted heads						
		Einkorn			Marquis			Kota
		Partial	Complete	Total	Partial	Complete	Total	Total
New Zealand	80	3.7[b]	1.1	4.8	1.0	0.3	1.3	34.5
Hungary[a]	65	9.5[c]	9.7	19.2	0.4	2.7	3.1	50.4
Norway	70	11.1	13.2	24.3	0.6	5.9	6.5	53.4
Sweden	80	9.9	1.9	11.8	1.8[b]	0.7	2.5	58.0
Manitoba	80	5.8	6.4	12.2	1.0	4.2	5.2	37.5
California	80	10.0	13.7	23.7	1.5	0.5	2.0	40.0
Washington	65	4.0	6.5	10.5	1.4	0.4	1.8	28.5
Uninoculated	—	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a] Hatvan.

[b] Individual heads lightly smutted.

[c] Individual heads almost completely smutted.

surance that they will be resistant in other geographical areas. Einkorn, C. I. 2433, for example, is comparatively resistant to form 1 of *T. tritici* from New Zealand, but is susceptible to form 2 from Norway. Gaines (20) also recently found that varieties which were resistant to bunt in one geographical area are not necessarily resistant in another. *T. tritici* from Germany was found to have different pathogenic capabilities from that which is common in eastern Washington. The American wheats were susceptible to the German form while the German wheats succumbed more readily to the American form.

There is, furthermore, no assurance that resistant varieties of wheat which have been developed for a particular locality will remain resistant over a long period of time. In the hard red spring wheat region Marquis and the durum wheats have been resistant to bunt prior to 1924. In subsequent seasons bunt has continued to be epidemic in the durums, so one many conclude that this outbreak has not been due to unusually favorable environmental conditions but rather to the occurrence of a new physiologic form in the region. Stephens (47) reported a similar occurrence of a new physiologic form of bunt in Oregon. Heretofore immune and highly resistant varieties of wheats, such as White Odessa, Martin, and Albit, suddenly became smutted in 1927. Since varieties of wheat resistant to bunt in one geographical area may not be resistant in another area, and since new forms are appearing in certain localities, it is evident that a study of physi-

TABLE 15.—*The percentage of smutted heads in Marquis, Kota, and Pentad wheats and in Einkorn inoculated with seven collections of Tilletia tritici at University Farm, St. Paul, Minn., in 1927*

Source of inoculum	Germination of chlamydospores in per cent	Percentage of smutted heads											
		Einkorn			Marquis			Kota			Pentad		
		Partial	Complete	Total	Partial	Complete	Total	Partial	Complete	Total	Partial	Complete	Total
New Zealand	60	21.9	1.5	23.4	1.3	0.2	1.5	5.6	4.1	9.7	6.8	0.4	7.2
Hungary[a]	65	48.4	3.3	51.7	3.6	0.3	3.9	8.7	23.1	31.8	7.8	0.9	8.7
Norway	75	50.8	6.7	57.5	7.5	5.8	13.3	4.9	25.0	29.9	6.3	3.9	10.2
Sweden	60	40.1	2.4	42.5	2.6	3.1	5.7	8.9	17.1	26.0	7.3	0.9	8.2
Manitoba	80	35.2	2.9	38.1	1.8	2.1	3.9	6.3	11.1	17.4	3.8	0.9	4.7
California	70	48.7	9.4	58.1	1.7	0.9	2.6	6.3	24.8	31.1	5.7	0.7	6.4
Washington	75	34.0	8.8	42.8	0.4	0.8	1.2	1.8	6.1	7.9	2.8	0.8	3.6
Uninoculated	—	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a] Hatvan.

TABLE 16.—*The relative susceptibility of Einkorn, Marquis, and Kota to four collections of Tilletia levis and to three of T. tritici at University Farm, St. Paul, Minn., in 1926*

Source of inoculum	Relative susceptibility[c]			Form number
	Einkorn	Marquis	Kota	
Tilletia levis				
Hungary[a]	R	R	S	1
Hungary[b]	R	M R	S	1
Minnesota	M R	M R	S	2
Egypt	M R	V R	M S	3
Tilletia tritici				
New Zealand	V R	V R	M S	1
Norway	M R	M R	S	2

[a] Hatvan.

[b] Debreizen.

[c] V R = very resistant; M R = moderately resistant; M S = moderately susceptible; S = susceptible.

ologic specialization as it affects the problem of breeding is of primary importance.

Pathogenicity of Physiologic forms of Ustilago hordei

Seven different physiologic forms of *U. hordei* were recognized in culture. It is important, of course, to know whether these forms differ in pathogenicity also. And particularly important is the question as to whether any of them are so virulent as to be able to infect varieties of barley that have been generally resistant. About 135 varieties and selections of barley were tested for their reaction to loose and covered smuts in 1924 to 1927. These varieties were heavily inoculated artificially with chlamydospores of *U. hordei*, but so little smut developed in most of them that the results were not considered significant. Observations were made also on the reaction of several varieties to the two smuts in other plots at University Farm, St. Paul, and at the University of Minnesota sub-stations located at Crookston, Grand Rapids, Morris, and Waseca. Lion, C. I. 923, which is very susceptible to loose smut, was found to be immune to covered smut. White Spring Hulless (C. I. not known) was found to be most susceptible to both the loose and covered smuts. Odessa, C. I. 182, Trebi, Minn. 448, Glabron, Minn. 445, and Svanhals, C. I. 187, may be classed as susceptible, while Manchuria, Minn. 184, and Svansota, Minn. 440, may be classed as resistant. The reaction of these varieties to smut was determined under Minnesota conditions. This does not mean, however, that they would

be resistant when grown in another geographical area. In fact Faris (16) distinguished four physiologic forms by differences in pathogenicity.

The writer made inoculations with two culturally distinct physiologic forms of *U. hordei* in order to determine if they could be differentiated on the basis of pathogenicity. In the spring of 1927 Lion barley, C. I. 923, Himalaya, C. I. 620, Manchuria, Minn. 184, Svansota, Minn. 440, Trebi, Minn. 448, and Wisconsin Pedigree, Minn. 454, were inoculated with sporidial cultures collected in Italy and at University Farm, St. Paul, Minn. The inoculum was grown in Erlenmeyer flasks on 2 per cent potato dextrose agar for a period of two weeks. Previous to inoculation the barley seed was treated with a 1:320 solution of formaldehyde, thoroughly washed in water and then dried. The seed was then allowed to germinate and inoculations made when the coleoptile had appeared by immersing the seed for a period of 20 hours in a suspension of sporidia in distilled water. All the seedlings were then sown on the same day in duplicated rod rows.

The results obtained from the inoculation of six varieties of barley with two forms of smut are summarized in table 17. No smut developed in Manchuria, Svansota, Trebi, or Wisconsin Pedigree when inoculated with form 2 which was collected at St. Paul, Minn. There were, however, differences in the virulence of the two forms on Himalaya and Lion. Himalaya proved to be susceptible to form 2 and highly resistant to form 1. On the other hand, Lion was susceptible to form 1 and immune to form 2. It is probable that there are many more forms which differ pathogenically.

Only two of the seven cultural forms were tested, but, not only were they pathogenically distinct, but one of them, the form from Italy, infected Lion barley which has been immune to *U. hordei* in Minnesota up to the present time.

Varietal Resistance of Oats to Ustilago levis and Ustilago avenae

In connection with the study of physiologic forms of oat smuts, a study was made of the varietal resistance of species and varieties of *Avena* to *U. levis* and *U. avenae*. The tests extended over a period of four seasons, during which 58 varieties belonging to six species of *Avena* were grown. Reed (34) and Reed and Griffiths (36) have reviewed the work of other investigators up to 1925. Reed (34) tested 154 agronomic strains and varieties of oats at the University of Missouri, which, when further tested by Reed and Griffiths (36) at widely separated stations, behaved in about the same way as they had in Missouri. Of the seven host species studied, *Avena brevis* Roth. and *Avena strigosa* Schreb., developed only smut-free plants. *Avena sterilis* L. was highly resistant. The other species were generally susceptible, although two varieties, Black Mesdag and Fulghum, were immune from both smuts.

In the writer's tests, the seed of each variety was sown in single rod rows at the rate of 15 grams to each row, this making about 300 plants to the row. Just before sowing, the seeds were uniformly inoculated with a mixture of chlamydospores of *U. levis* and *U. avenae*. Percentages of smut were obtained by counting the number of diseased heads instead of diseased plants. As the two smuts can be considered as one for practical purposes, all smutted heads were counted without distinguishing between the two kinds of smut.

The data on varietal resistance at University Farm are summarized in table 18. All of the averages in table 18 are not strictly comparable, because in a few instances varieties were not tested all four seasons, but they are valuable in that they do furnish an indication of resistance and susceptibility. With few exceptions the results obtained confirm those of previous investigators. *A. nuda* was found to be extremely susceptible, *A. strigosa* highly resistant, and *A. brevis* immune. With one exception the varieties of *A. sterilis* were resistant. In Iowa Burt (C. I. number not known) an average of 5.4 per cent of smut developed. Most of the varieties of *A. sativa* and *A. sativa orientalis* were susceptible. A few, however, were either immune or highly resistant. Black Mesdag, C. I. 1877, and Markton, C. I. 2053, were immune throughout the four seasons. Early Champion, C. I. 1930, was the most susceptible variety of this group, it having an average of 29.9 per cent of smut. Black Mesdag, which was found to be immune by other investigators, also was immune in the writer's tests. Golden Giant, C. I. 1606, was immune in all tests when inoculated with both smuts. In Reed's tests (34), however, this variety proved to be susceptible when inoculated with smut in Missouri. In this variety he obtained 16.0 per cent of *U. avenae* and 45.6 per cent of *U. levis*. This difference is undoubtedly due to the presence of a different physiologic form of the fungus in Missouri than the one prevalent at University Farm, St. Paul, Minn., as Reed (35), in a later publication, has shown that there are physiologic forms of the oat smuts which differ in their pathogenic capabilities.

Five physiologic forms of *U. levis* and eighteen of *U. avenae* were differentiated on the basis of cultural characteristics. An attempt was made to determine whether these forms differed also in their pathogenic capabilities and whether varieties of oats that had proven immune or highly resistant to smut at University Farm would be attacked by any of these physiologic forms. Ten varieties of oats were inoculated individually with sporidia of three physiologic forms of *U. levis* and five forms of *U. avenae* as described in similar tests with *U. hordei*. Unfortunately, however, no results were obtained, since smut failed to develop in any of the varieties. Evidently the technique was faulty, but in what respect has not yet been determined.

TABLE 17.—*Summary of data on tests for pathogenicity of two physiologic forms of Ustilago hordei at University Farm, St. Paul, Minn., in 1927*

Variety	Smut from Italy					Smut from St. Paul					Uninoculated
	Series A		Series B		Average per cent infected	Series A		Series B		Average per cent infected	
	Total no. heads	Per cent infected	Total no. heads	Per cent infected		Total no. heads	Per cent infected	Total no. heads	Per cent infected		
Lion	515	8.8	412	5.6	7.2	489	0.0	502	0.0	0.0	0.0
Himalaya	390	Tr.	409	0.0	Tr.	246	21.5	311	12.5	17.0	0.0
Manchuria	479	0.0	502	0.0	0.0	438	Tr.	461	0.0	Tr.	0.0
Svansota	480	0.0	496	0.0	0.0	468	Tr.	492	0.0	Tr.	0.0
Trebi	460	0.0	413	0.0	0.0	481	Tr.	399	0.0	Tr.	0.0
Wisc. Ped.	417	0.0	391	0.0	0.0	397	Tr.	431	Tr.	Tr.	0.0

TABLE 18.—*The percentage of smut (Ustilago avenae and U. levis) on oat varieties at University Farm, St. Paul, Minnesota, in 1924–1927*

Species and varieties	C. I. number	Year and percentage of smutted heads				
		1924	1925	1926	1927	Average
Avena nuda		68.0	—	—	16.2	42.1
A. strigosa		0.0	0.0	1.0	0.0	Tr.
A. brevis		0.0	0.0	0.0	0.0	0.0
A. sterilis						
Burt		0.0	1.1	0.0	0.0	Tr.
California Burt		1.5	1.2	3.9	1.4	2.0
Iowa Burt		5.0	5.0	7.8	3.8	5.4
King		0.0	1.0	Tr.	Tr.	Tr.
Red Rustproof	1356	0.0	2.9	0.0	Tr.	0.7
A. sativa						
Anthony (Minn. 686)		2.0	1.3	8.3	11.0	5.8
Aurora	831	3.0	15.9	8.8	9.7	9.4
Awnless Probsteier	1888	1.0	8.7	4.5	7.8	5.5
Belyak	1630	0.0	0.6	1.5	5.0	1.8
Black Mesdag	1877	0.0	0.0	0.0	0.0	0.0
Black Norway		0.0	3.5	2.0	2.5	2.0
Canadian	1625	1.0	7.2	11.0	4.0	5.8
C. I. 602		1.0	3.0	5.5	11.4	5.2
C. I. 603		0.0	4.0	2.5	3.6	2.5
C. I. 606		1.0	0.4	Tr.	0.7	0.5
C. I. 620		1.0	16.5	17.0	6.8	10.1
Culberson	273	0.0	8.6	3.0	2.0	3.4
Danish Island	519	1.0	6.2	9.0	16.4	8.2
Early Champion	1930	8.0	54.6	30.5	26.6	29.9
Early Dakota		1.0	2.8	11.0	11.2	6.5
Early Gothland	1723	1.0	2.5	4.5	7.2	3.8
Garton 473	1613	1.0	3.0	3.5	1.4	2.2
Garton 691		5.0	0.0	5.0	1.8	3.0
Golden Drop	1890	0.0	7.2	6.3	8.4	5.5
Gopher	2027	0.5	23.2	8.0	3.8	8.9
Green Russian		1.0	1.5	4.5	2.4	2.4
Irish Victor	1896	1.0	Tr.	5.0	5.6	2.9
Japan Selection	1889	2.0	3.5	11.5	29.6	11.7
Joanette	1762	1.0	14.9	9.9	7.8	8.4
June	1902	3.0	11.4	9.0	8.6	8.0
Kanota	839	4.0	4.6	8.5	2.6	4.9
Kherson	459	0.0	24.8	20.0	13.2	14.5
Kherson Selection	1905	1.0	35.5	17.5	18.4	18.1
Lincoln	1463	1.0	18.2	6.0	7.4	8.2
Markton	2053	0.0	0.0	0.0	0.0	0.0
Minota	1285	1.5	20.2	18.0	11.0	12.7
Monarch	1682	0.0	6.2	2.5	4.6	3.3
Monarch Selection	1879	1.9	2.4	4.5	7.0	4.0
North Finnish	1882	0.0	3.6	8.0	8.0	4.9
Old Island Black	1881	0.0	17.6	9.8	7.4	8.7
Scottish Chief	1901	0.0	0.0	2.8	1.8	1.2
Silvermine	1629	0.0	0.0	0.5	5.4	1.5
Silvermine Selection	1894	0.0	0.0	1.5	4.8	1.6
Sixty Day	826	0.5	26.9	3.0	8.6	9.8
Sixty Day Selection	1906	0.0	10.6	0.5	2.8	3.5
Swedish Select	802	0.0	8.6	6.0	7.6	5.6
Tobolsk	1709	1.0	3.6	4.0	7.4	4.0
Victor	803	2.0	10.0	18.0	12.2	10.6

TABLE 18.—*Continued*

Species and varieties	C. I. number	Year and percentage of smutted heads				
		1924	1925	1926	1927	Average
A. sativa orientalis						
Black Tartarian		0.0	—	16.0	6.8	7.6
Garton 585	1868	0.0	0.6	1.0	1.0	0.7
Garton 748		0.0	0.0	6.0	13.4	4.9
Garton 784		5.0	6.0	11.0	3.2	6.3
Garton Gray		0.0	—	11.5	4.2	5.2
Green Mountain	1872	2.0	3.6	5.0	7.8	4.6
Golden Giant	1606	0.0	0.0	0.0	0.0	0.0
Sparrowbill	1604	3.0	1.5	11.5	3.0	4.8
Storm King	1602	3.0	1.8	8.0	0.6	3.4
White Tartar	1614	0.0	28.2	6.0	7.2	10.4

DISCUSSION AND CONCLUSIONS

There are many physiologic forms of the cereal smut fungi. They differ greatly in cultural characteristics, in physico-chemical relations and, in some cases at least, in pathogenicity. On culture media the physiologic forms differed strikingly from each other in the following characters: color, topography of colonies, character of surface, consistency, and type of margin. The cultural differences can be detected only when the proper differential materials are used. There may be decided differences between forms grown under one set of environmental conditions which may not be apparent when the same forms are grown in another environment. Under identical conditions, however, the cultural characteristics of given forms are remarkably constant. The differences in the cultural characteristics of some of the physiologic forms are so great that one might even consider them as different species. On the other hand, the cultural characteristics of forms of different species are sometimes strikingly similar.

Twelve distinct forms of *U. nuda* and fourteen of *U. tritici* were distinguished on culture media. At present these two smuts are considered as distinct species. It is significant, however, that some of the forms of *U. nuda* resemble some of *U. tritici* more closely than they do certain other forms of *U. nuda*. The chlamydospores of the two smuts are alike, neither produces sporidia, and the effect on their respective host plants is the same. It would seem, then, that *U. nuda* and *U. tritici* are themselves nothing more than physiologic forms. The principal difference between them is that *U. nuda* infects barley and *U. tritici* infects wheat. It is quite possible, however, that this specialization does not always occur. In fact Humphrey and Tapke (23) have shown that *U. tritici* can infect rye. The writer considers it probable that some of the forms of *U. nuda* probably will infect wheat, and some of *U. tritici* probably will infect barley.

It is possible also that *U. levis* and *U. avenae* are physiologic forms of the same species. Five physiologic forms of *U. levis* and eighteen forms of *U. avenae* were distinguished on culture media. Here again the differences between certain forms of the same species were greater than those between some of *U. levis* and *U. avenae*. These two smuts have the same life history, they apparently have the same host range, the spores are of the same size and shape, and both are light on one side; in most cases it is impossible to tell the two apart. Furthermore, Reed (34) found that various strains and varieties responded similarly to inoculation with the two smuts. At present the two species are differentiated on the basis of markings on the chlamydospores walls. However, in a miscroscopic examination of the chlamydospores from a single head supposedly infected with *U. avenae*, all gradations in degree of echinulate markings, from practically smooth to highly echinulate, may be found.

Not only do the physiologic forms of some of the smuts differ in general appearance on culture media but they react quite differently to temperature. Physiologic forms of *U. tritici, U. nuda,* and *U. hordei* were differentiated on the basis of differences in optimum temperature for growth, as measured by the size of colonies and also by the amount of radial growth attained at certain temperatures. The fact that physiologic forms of smuts do react differently to temperature might very easily account for the development of characteristic physiologic forms in certain geographical areas. Furthermore, if these forms which differ in their reaction to temperature also differ in their ability to attack certain varieties, it would account, in part at least, for the variation from year to year in the susceptibility of certain varieties to smut. Not only do the physiologic forms differ in general appearance on culture media and in their physico-chemical reaction but some also differ pathogenically.

Physiologic forms of *U. hordei* were found to differ in their ability to infect certain varieties of barley. One form was very virulent on a variety that had always been immune to covered smut in Minnesota. On the other hand, it was only weakly pathogenic on a variety which is susceptible to a form of *U. hordei* collected at University Farm, St. Paul, Minn.

In a study of the varietal resistance of spring wheats to bunt caused by *T. levis*, the durum wheats as a class were found to be more resistant than the common wheats. There were, however, wide differences within both classes, ranging from highly resistant varieties to those completely susceptible. Resistance then is not correlated with any particular morphologic type and it should be possible to breed varieties of wheat having desirable agronomic characteristics which are highly resistant to bunt. This problem is complicated, however, by the fact that there are physiologic forms of the pathogenes causing bunt. Three physiologic forms of *T. levis* and

two of *T. tritici* differed in their parasitic capabilities on several varieties of wheat. It seems likely that several more forms could have been distinguished had more differential hosts been used. One of the forms of *T. tritici* is characterized by being relatively virulent on all the varieties tested while another form is only weakly parasitic or moderately virulent on the same varieties. There also are distinct differences in the virulence of the physiologic forms of *T. levis*.

The present studies have shown that there are numerous physiologic forms in the cereal smut fungi and that they are widely distributed throughout different geographical areas. Some of these physiologic forms differ in their pathogenic capabilities, and varieties which are immune or highly resistant to smut in one locality may be susceptible in another. As a result of this condition, breeding for smut resistance becomes more complicated and requires a thorough study of the number, distribution, and pathogenic capabilities of the physiologic forms of the pathogene. New physiologic forms of the smut fungi probably are being formed constantly, either by hybridization or mutation. Stakman and Christensen (45) have shown that inoculation with two strains of opposite sex is necessary in order that corn may become infected with *U. zeae*. Hybridization takes place in or on the corn plant, which means that new combinations result. A new physiologic form may then be produced as a result of hybridization. Dickinson (11 and 12) has shown that when oat seedlings are inoculated with sporidia of *U. levis* of one gender, (A) or (B), infection will not take place. When, however, they are inoculated with hyphae resulting from the fusion of sporidia of two genders, (A) and (B), or from hyphae which result from a fusion between sporidia of *U. levis* and *U. hordei*, infection will take place. Recombination and segregation may occur here also, with the result that a new physiologic form may be formed. Christensen and Stakman (9) found mutations to occur frequently in *U. zeae*. These mutants differed from their parents not only in general appearance but also in pathogenicity. Just how the sectors of *U. hordei* and *U. avenae* arose can not be definitely stated, but the significant thing is that new physiologic forms are being formed in the cereal smut fungi. If they differ in their pathogenicity as do many of the forms which are already present, which is very likely, then breeding for smut resistance even as a regional problem becomes a continuous one.

SUMMARY

1. *Ustilago tritici, U. nuda, U. hordei, U. levis, U. avenae, Tilletia levis,* and *T. tritici* are group species consisting of many physiologic forms. The physiologic forms are widespread in the United States and foreign countries.

2. Fourteen physiologic forms of *U. tritici*, twelve of *U. nuda*, seven of *U. hordei*, five of *U. levis* and eighteen of *U. avenae* were studied in detail. The forms can be distinguished in culture by the following characters: color, topography, surface, consistency, and type of margin.

3. The differences between physiologic forms in culture sometimes are very great but they can be distinguished only when grown on the proper media. When grown under identical conditions the cultural characteristics of the physiologic forms are constant.

4. The differences in cultural characteristics of different forms of *U. nuda* are sometimes far greater than those between the so-called species *U. tritici* and *U. nuda*. The morphological differences between these two fungi are scarcely pronounced enough to consider them as species and they are considered by the writer as physiologic forms.

5. The differences in cultural characteristics of different forms of *U. levis* are sometimes far greater than those between the so-called specis of *U. levis* and *U. avenae*. It is considered by the writer that these are possibly nothing but physiologic forms.

6. Some of the physiologic forms of *U. tritici*, *U. nuda*, and *U. hordei* differ also in their physico-chemical reactions. Differences were noted in the optimum temperatures for growth and in the amount of growth, measured by the diameter of the colonies, at different temperatures.

7. When subjected to different temperatures, physiologic forms of *U. nuda* respond differently with respect to the formation of counter-clockwise growth of mycelium.

8. Oat varieties varied greatly in their resistance to smut. Three varieties, Markton, Black Mesdag and Golden Giant, and *Avena brevis* were immune. *A. strigosa* and varieties of *A. sterilis* were in general resistant. Most of the varieties of *A. sativa* and *A. sativa orientalis* were susceptible.

9. Wheat varieties varied greatly in their resistance to bunt; none were immune. The durums as a class are more resistant than the common types. The common wheats possess all gradations of resistance and susceptibility.

10. Two physiologic forms of *Ustilago hordei* can be recognized by their parasitic behavior on Lion and Himalaya barley. Lion is immune to the forms prevalent in Minnesota and susceptible to an Italian form. Himalaya is highly resistant to the same Italian form but susceptible to the Minnesota form.

11. *Tilletia levis* and *T. tritici* both comprise distinct physiologic forms which can be recognized by their degree of virulence on Kota, Marquis, and Pentad wheats and on Einkorn.

12. Collections of *T. levis* were obtained from Minnesota, Italy, Egypt, and two localities in Hungary. There were at least three physiologic forms

in these collections: one from Minnesota, one from Hungary, and one from Egypt.

13. Collections of *T. tritici* were obtained from New Zealand, Hungary, Norway, Sweden, Canada (Manitoba), and from Minnesota, California, and Washington in the United States. Two forms can be recognized readily: a virulent one from Norway and a relatively weak one from New Zealand.

14. It seems likely that a considerable number of forms both of *T. levis* and *T. tritici* can be distinguished if the proper differential hosts are used.

15. Sectors occurred in cultures of *U. hordei* and *U. avenae* and differed from the parents in color and rate of growth.

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ON THE OCCURRENCE OF PYCNIA AND AECIA IN CERTAIN RUST FUNGI[1]

J. H. Craigie

Evidence secured by experimentation in the greenhouse has demonstrated that *Puccinia helianthi* Schw. and *P. graminis* Pers. are heterothallic; that the majority, at least, of pustules derived from infections by single sporidia of these rusts never produce aecia;[2] and that when pycniospore-containing nectar of such pustules is transferred from one to another, so that a thorough mixing is effected, aecia appear in a few days.[3] A few additional observations and experiments bearing on the problem of sex in rust fungi are set forth in this paper.

For the sake of convenience in what follows, pustules of monosporidial origin are designated as "simple" pustules, and those of bisporidial origin as "compound" pustules.

EVIDENCE OF HETEROTHALLISM IN PUCCINIA GRAMINIS

It was intimated in one of the previous papers[3] that *Puccinia graminis* is heterothallic, but, as that paper treated specifically of the function of the pycnia of rusts, no data were furnished to show the relative numbers of compound pustules and simple pustules which did, and which did not, produce aecia. A statement concerning this point will first be made.

Both simple and compound pustules of *P. graminis* were obtained on young leaves of barberry (*Berberis vulgaris*) by sowing sparsely the sporidia of germinating teliospores over the surface of the leaves. Figure 1 shows a barberry leaf which was photographed 20 days after it was inoculated. A compound pustule bearing aecia appears on the left-hand side of the midrib. On the right-hand side are three simple pustules in which no aecia have developed. The pitted appearance of these three pustules is due to the formation of haploid mycelial wefts which develop in the simple pustules just underneath the epidermis, and simulate in general contour that of a young aecium. They never break through the epidermis, however, and rarely, if ever, of themselves produce aeciospores. This point will be discussed in a subsequent paper.

[1] Contribution from the Division of Botany, Dominion Experimental Farms Branch, Ottawa.

[2] Craigie, J. H. Experiments on Sex in Rust Fungi. Nature 120: 116–117. 1927.

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The pustules first appeared as tiny dots. As soon as they became evident, the position on the leaf of each individual pustule, and of each pair of pustules which might be expected to coalesce later, was mapped on a label which was then attached to the leaf. In this way it was possible to tell, when the pustules became older, which pustules were simple, and which were compound. If the distance between a pair of pustules was greater than 4 mm., each of the components was considered as a simple pustule, as the two seldom achieved a thorough coalescence. Usually the points at which infections took place on the leaf were easily discernible, but sometimes two infections occurred so close together that it was extremely difficult to decide whether or not there were two infections. This was an ever-recurring difficulty. Where there was any doubt, the pustules were always classed as simple. For this reason certain pustules which were very probably compound were tabulated as simple pustules.

As the number of barberry plants available and suitable at any one time for inoculation purposes was limited, the inoculating was done at different

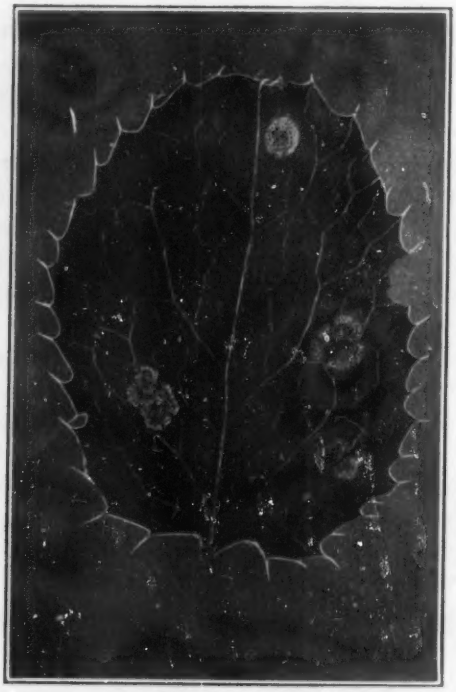

FIG. 1. Under side of a barberry leaf showing one compound pustule with aecia, on left of midrib, and three simple pustules without aecia, on right of midrib. Photographed 20 days after inoculation. ×2.

times. The data were recorded 20 days after each inoculation. Before the end of that time, the majority of the paired pustules, designated as compound, had completely coalesced, and where the paired pustules, or components, were of opposite sex, aecia had appeared. In a few of the compound pustules in which the components were relatively far apart, coalescence was not yet thoroughly accomplished. The results are summarized in table 1.

TABLE 1.—*Number of simple and compound pustules of Puccinia graminis on leaves of Berberis vulgaris in which aecia had developed and in which aecia had not developed 20 days after inoculation*

Kind of pustule	Total no. of pustules	No. of pustules	
		with aecia	without aecia
Simple (monosporidial in origin)	174	11	163
Compound (bisporidial in origin)	59	24	35

It should be mentioned that these data were collected before the function of the pycnia was discovered, and it is quite possible that, in at least some of the 11 simple pustules which gave rise to aecia, the development was induced by the transfer to them of nectar from pustules of opposite sex, by insects or other agencies. It is possible also that a few others, although tabulated as simple pustules, were really bisporidial in origin.

From time to time after the data recorded in table 1 were taken, some pustules of both types, which had hitherto produced no aecia, developed aecia. The appearance of aecia in a few of these compound pustules may be explained by the fact that the two components of each pustule, although of opposite sex, were at first rather widely separated (about 4 mm.), and consequently the interaction of their mycelia was delayed. The belated development of aecia which occurred in the other compound pustules and in the simple pustules was probably induced, at least to some extent, by a fortuitous transference to them of nectar from pustules of opposite sex. In some of the pustules aecia may have arisen spontaneously, but doubt is thrown on this possibility by the results obtained with simple pustules of *P. helianthi* recorded elsewhere in this paper.

Many of the pustules, both simple and compound, which did not produce aecia persisted for eight or nine weeks, some even for a longer time. When the experiment finally terminated, it was found that of the 174 simple pustules and the 59 compound pustules, 117 of the former and 23 of the latter had failed to develop aecia, a result which, after due allowance is made for the imperfections of the experiment, is fairly conclusive evidence of the heterothallic nature of this rust.

AECIA IN SIMPLE PUSTULES

When the effect of mixing the nectar of simple pustules of opposite sex was discovered, suspicion arose concerning the ability of such pustules to produce aecia spontaneously. In the early stages of the investigation, approximately 40 per cent of these pustules developed aecia, but later, when the plants bearing the pustules were kept in cages, aecia appeared in a very much smaller number. The inference was drawn that quite possibly, in the frequent examination of these pustules—the leaves were turned over by thumb and fore-finger, the nectar of (−) pustules was occasionally carried to (+) pustules, and *vice versa,* thus inducing the development of aecia in some pustules. Moreover, no protection was afforded these pustules against the visitation of flies or other insects which might by chance have entered the greenhouse.

In order to get more exact information as to whether simple pustules were capable in themselves of producing aecia, the following experiment was undertaken. Leaves of sunflower seedlings growing in the greenhouse were inoculated with sporidia of *Puccinia helianthi* when the two first true leaves were about one inch or slightly less in length. The sporidia were allowed to fall sparsely on to the upper surface of the leaves.

Usually about six seedlings grew in a pot, but, whenever the minute pustules appeared, each plant showing infection was transplanted alone in a separate pot and covered with a screen wire cage. It was thought that by keeping each plant in an individual cage the opportunity for the transfer of nectar by any insect which might enter the cage would be reduced to a minimum. In general, not more than one pustule occurred on any individual leaf. Indeed, many of the plants bore but a single pustule. Infrequently, however, two or more occurred on the same leaf. All uninfected leaves were removed from the plants and no new ones were allowed to develop.

With the exception of a few compound pustules which were readily discernible, and a small number of pustules which were marked as simple but which produced aecia within 12 or 14 days, all the others were apparently simple. Those which were at first marked as simple but developed aecia within two weeks from the time of inoculation possibly arose from two infections which took place so close together that the two infections appeared as one. Sometimes it was difficult to determine with certainty whether a single sporidum caused the infection, or whether it arose from two sporidia which alighted side by side on the leaf. It can not be said with confidence that these apparently simple pustules were in reality compound, but the likelihood is that they were. In order to give any compound pustules which might have had components of opposite sex an opportunity

to develop aecia and to avoid dealing with doubtful cases, no data were recorded until the pustules were 17 days old. There were altogether 228 pustules, all apparently simple ones, in which no aecia at all were evident. Of these, 93 were borne singly, one on each plant; of the remaining 135, usually two, but sometimes three or even four, occurred on the same plant. None of the pustules which developed on the cotyledons were considered, for the majority of the cotyledons became chlorotic and died comparatively early in the experiment.

Not all of the pustules persisted for the same length of time. Some became necrotic during the fourth week; most of them died before the end of the sixth week; but a few were still living and exuding nectar in their peripheral region when seven weeks old. The experiment terminated at the end of the seventh week. Within that time, aecia appeared in 11 of the 228 pustules. There was no regularity in their time of appearance, as if in response to a common stimulus. First one pustule—then a few days or a week later another pustule — would give rise to aecia, as if influenced by a separate and distinct stimulus. The results are summarized in table 2.

TABLE 2.—*Number of simple pustules of Puccinia helianthi, over 17 days old, which produced aecia and which did not produce aecia*

Pustules per plant	No. of pustules which produced	
	Aecia	No aecia
One	2	91
More than one	9	126
Total	11	217

From the time the pustules first appeared, an examination of each plant was made daily in order to destroy any insect which might have found its way through the mesh of the cages and thus gained access to the plant. White flies (*Aleyrodes*) and thrips (*Heliothrips*) were about the only ones which succeeded in doing this. Any of these that were found were removed and destroyed. The former were rarely ever seen in contact with the pustules, but the latter showed a decided preference for nectar as a diet. In spite, therefore, of considerable precautions, there was the possibility, especially in those cages in which the plants bore more than one pustule, that, during the seven weeks over which the experiment extended, some slight mixing of nectar took place.

Possibly, with the absolute exclusion of insects, or other means by which nectar might be transferred from one pustule to another, aecia would have arisen in none of these pustules. This, however, was not demonstrated.

At present, it is impossible to say that aecia do not arise spontaneously in pustules of monosporidial origin, but it has been clearly shown that, under the conditions of the experiment, only a very small percentage of such pustules did give rise to aecia. It is desirable that this point should be definitely settled, but to do so would require very rigid control and many more pustules than the writer can hope to have available this year.

OCCURRENCE UNDER NATURAL CONDITIONS OF PYCNIA UNACCOMPANIED BY AECIA

So far as the writer is aware, it is not recorded that pustules of monosporidial origin occur in nature, although undoubtedly they have been seen by other observers and mistaken by them for young pustules in which aecia had not yet developed.

From observations made during the summer of 1927 and of 1928, it has been found that in nature pycnia are frequently unaccompanied by aecia in the following rusts: *Puccinia graminis*, on *Berberis vulgaris* var. *purpurea*; *P. coronata* Cda., on *Rhamnus cathartica*; *P. pringsheimiana* Kleb., on *Ribes grossularia* (cultivated and wild); and a *Gymnosporangium* sp. (possibly *corniculans* Kern.), on *Amelanchier alnifolia*. Very probably the phenomenon occurs under natural conditions in other rusts also, but, as those mentioned were the most accessible, observations were confined mostly to them.

Leaves of these hosts bearing young and apparently simple pustules were marked by means of a small tag as soon as the pustules were noticed. When the pustules were about 14 or 15 days old, those which showed no evidence at all of aecia were selected for further observation. It was thought that if any of the first marked pustules originated from two contiguous infections and the mycelia of the two were of opposite sex, aecia would appear in such compound pustules within this time; if the mycelia were of the same sex, no aecia would be expected. In all the carefully examined pustules, pycnia were invariably present.

From time to time during the next three or four weeks, these pustules were examined. Within that time, some of the pustules produced aecia, but others did not. All of the pustules which produced aecia and most of those that produced none became necrotic, but a few of the latter persisted fresh-looking and vigorous for a week or more longer. The results of these observations for 1927 are given in table 3.

It should be noted that none of these pustules were protected in any way from the visitation of insects, and there is little doubt that through their agency the transfer of nectar from one pustule to another took place, as insects of various kinds were seen flying about or crawling over the leaves

TABLE 3.—*Summary of observations made in 1927 on the occurrence in nature of aecia in simple pustules*

Name of rust	No. of pustules 14 days old	Observation period (weeks)	No. of pustules at end of period	
			with aecia	without aecia
Puccinia graminis	50	4½	37	13
P. coronata	61	2½	45	15
P. pringsheimiana	60	3	16	44
Gymnosporangium sp.	60	3	52	8

of the host plants, some even in contact with the pustules. However, it should also be noted that, on account of the excessive precipitation, the pustules were frequently washed, and only very seldom was there any noticeable amount of nectar available for transfer. This fact accounts, perhaps, for the failure of so many of the pustules to produce aecia, despite the activity of the insects.

During the summer of 1928, young pustules which were apparently monosporous in origin were again marked, but instead of leaving them exposed, as was done in the previous year, most of the leaves bearing them were covered with one ply of white cheese-cloth, in order to intercept as far as possible the visitation of insects. This protection excluded fairly effectively most of the winged insects, but not so well, perhaps, ants and small spiders.

Not all the pustules marked were found as soon as they appeared. At the time the coverings were applied, some would be at least one week old; most of them, however, were just appearing, or not more than two or three days old. Unfortunately, the pustules available for study were much less numerous than one could have wished. Practically no infection occurred on *Rhamnus cathartica* at the Agricultural College, Winnipeg. None at all were found on *Amelanchier alnifolia*. Only a few appeared on *Berberis vulgaris*, and the pustules on *Ribes grossularia* were relatively scarce.

Certain of the pustules on *Ribes* were left unprotected in order to serve as a check for the protected ones, and for comparison with the data collected the previous year. The results are summarized in table 4.

It will be seen from tables 3 and 4 that a much smaller percentage of the covered pustules in 1928 produced aecia than of the uncovered ones in 1927; and that, in 1928, less than 20 per cent of the covered pustules of *P. pringsheimiana* developed aecia compared with 50 per cent of the ones which were not covered. Apparently, by preventing the free access of insects to the pustules, the opportunities for transferring the nectar of one

TABLE 4.—*Summary of observations made in 1928 on the occurrence in nature of aecia in simple pustules*

Name of rust	No. of pustules	Observation period (weeks)	No. of pustules at end of period	
			with aecia	without aecia
Puccinia graminis (covered)	20	3	4	16
P. coronata (do)	2	3	0	2
P. pringsheimiana (do)	33	3	6	27
do (uncovered)	18	3	6	12

pustule to another were reduced, and, consequently, the number of simple pustules which remained free of aecia was augmented. It is not probable that the cheese-cloth covering intercepted sufficient sunlight to inhibit or retard materially the formation of aecia. This supposition is supported by the fact that a certain number of the covered ones produced aecia. Very probably the development of aecia in these pustules was induced by the

FIG. 2. Under side of a barberry leaf showing aecia present on the left-hand side, but not on the right-hand side, of a pustule of *P. graminis* which arose from an infection by natural inoculation. ×2.

transfer of nectar by insects before the covering was applied. This of course is but conjecture.

From the fact that simple pustules of *P. coronata*, of *P. pringsheimiana*, and of the *Gymnosporangium* sp. behave under natural conditions as do similar pustules of *P. graminis*, and as *P. graminis* has been shown by experiments in the greenhouse to be heterothallic, it may be inferred that these three rusts also are heterothallic.

While the data recorded above were being collected, it was noticed that in a few of the pustules which had not previously developed aecia, aecia began to appear at one side or in one section of a pustule. This phenomenon was observed in simple pustules of *P. coronata*, of *P. pringsheimiana*, and of *P. graminis* (Fig. 2). A plausible explanation of this behavior is that the nectar from pustules of opposite sex had been deposited on the upper surface of those sections only of the pustules, and as a result aecia had developed.

Using this interpretation as a cue, an attempt was made to reproduce similar results in the greenhouse in simple pustules of *P. helianthi*. The nectar from a dozen or more pustules of this rust was collected and deposited in one drop. By so doing it was hoped that some nectar would be gathered

FIG. 3. Under side of a barberry leaf which was artificially inoculated in the greenhouse, showing aecia present on the right-hand side, but not on the left-hand side, of a simple pustule of *P. graminis*. When the pustule was 20 days old, a small amount of composite nectar was applied to the upper surface of that section which now bears the aecia. The photograph was taken 7 days after the nectar was applied. ×2¼.

from pustules of opposite sex and that the drop would contain both (+) and (−) pycniospores. Small amounts of this composite nectar were applied to one side only of the simple pustules when they were approximately four weeks old. In each case, aecia developed within four or five days on the under surface of the pustule directly beneath the section to which the nectar was applied. The experiment was repeated with simple pustules of *P. graminis,* three and one half weeks old, with similar results (Fig. 3).

The exudation of nectar is much more copious in pustules of *P. graminis* than in pustules of *P. helianthi.* Therefore, before the composite nectar was applied to any pustule of *P. graminis,* as much as possible of the nectar which it had itself produced was drawn off by means of a capillary tube. In most of the pustules the aecia remained localized, particularly in those of *P. helianthi*; but in some pustules of *P. graminis* there appeared to be a tendency for aecia, after they had formed in the treated section, to develop belatedly in the other part of the pustule—the course of development being from the treated section toward the opposite edge.

It is difficult to say to just what cause this belated formation of aecia was due. Possibly, in the course of a few days the pycniospores were carried slowly across the surfaces of the pustules by flowing movements of the nectar, owing to the fact that the pustules are usually convex or concave, rarely flat, and day by day as more nectar accumulated, adjustments in the level of the nectar took place, thus bringing the composite nectar into contact with pycnia in the untreated section of the pustule. This explanation seems to be supported by the fact that in pustules of *P. helianthi,* where, under greenhouse conditions, the amount of nectar exuded is comparatively small, aecial development was confined almost entirely to the sections treated. Another possible explanation for the development of belated aecia in the untreated sections of these pustules is that diploid mycelia may have gradually extended from the treated into the untreated parts and there given rise to aecia.

The occurrence in nature of aecia in one sector, or one part, of a simple pustule and the duplication of a similar phenomenon by experimental methods in the greenhouse are of interest in themselves, but they are mentioned chiefly to lend additional support to the evidence already given that *P. coronata* and *P. pringsheimiana* are heterothallic. In appearance and behavior they simulate the two rusts which have been shown by experimentation to be heterothallic.

SUMMARY

1. Experiments carried out in the greenhouse have shown that *Puccinia graminis* is heterothallic.

2. Experimentation has shown that, under greenhouse conditions, aecia develop spontaneously in few, if any, simple pustules of *Puccinia helianthi*.

3. Observations have shown that simple pustules of *Puccinia coronata*, of *P. pringsheimiana*, of a *Gymnosporangium* sp., and of *P. graminis* occur under natural conditions on their respective aecial hosts. This fact indicates that, in addition to *P. helianthi* and *P. graminis*, these three other rusts also are heterothallic.

4. Pustules of *P. coronata* and *P. pringsheimiana*, as well as of *P. graminis*, have been found in nature bearing aecia in only one sector, or part, of each pustule. Experimentation in the greenhouse with simple pustules of *P. graminis* and *P. helianthi* has reproduced similar conditions, a result which further indicates that *P. coronata* and *P. pringsheimiana* are heterothallic.

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A SIMPLE APPARATUS FOR ISOLATING SINGLE SPORES[1]

W. F. Hanna

The securing of monosporous cultures of fungi has become a very important preliminary part of many mycological and phytopathological investigations. Several useful methods of isolating single spores have been described. Very few of these methods have come into general use, either because they can be employed only under certain conditions, or because of the high degree of technical skill required in their use. Elaborate micromanipulators designed for even the most delicate work may be obtained, but they are rather expensive and are not to be found in most biological laboratories. Any efficient and simple apparatus which may be constructed in the laboratory, therefore, should be of general interest.

In a recent paper, Dickinson[2] described a method of isolating individual spores and bacteria. According to this method, a thin film of agar is placed on the surface of a cover-glass and the spores to be isolated are applied to this agar film. The cover-glass is then inverted over a Van Tieghem cell, and the latter placed on a glass slide under the objective of the microscope. Through an opening in the front of the Van Tieghem cell is inserted the arm of the "isolator," to the end of which a glass needle is attached, so that its point is directed upward to the surface of the agar. By means of a suitable mechanical device, the point of the needle may be moved up to any spot on the surface of the agar. When a particular spore is to be separated from its fellows, the tip of the needle is brought upward until it comes in contact with the film of moisture on the surface of the agar, immediately below the spot where the spore is resting. At this point, a cone of liquid is formed between the tip of the needle and the surface of the agar. By a lateral movement of the needle, the spore is carried along in the cone of liquid to the margin of the film of agar. When the needle is lowered, the cone of liquid is broken, and the spore is thus left on the surface of the agar at a convenient distance from all other spores. The point where the spore lies is now marked. Later, the spore and the agar about it may be removed by means of a sterile instrument and transferred to fresh medium.

Dickinson has also suggested that, with a needle of suitable size, the spore may be removed on the point of the needle when the cone of liquid is

[1] Published with the Approval of the Director, No. 798 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] Dickinson, S. A method of isolating and handling individual spores and bacteria. Proc. Roy. Soc. Medicine 19: 1–4. 1926.

broken. In practice, I have found that this method of removing individual sporidia from the promycelia of *Ustilago zeae* and *Sorosporium reilianum* is highly satisfactory. Without question, the method may be employed in isolating single spores of almost all species of fungi. The apparatus to be described was used in isolating sporidia by this method. It serves the purpose quite as well as an expensive manipulator and possesses the advantage of being easily constructed from materials present in many laboratories.

Figure 1 shows the apparatus as it appears when attached to the stage

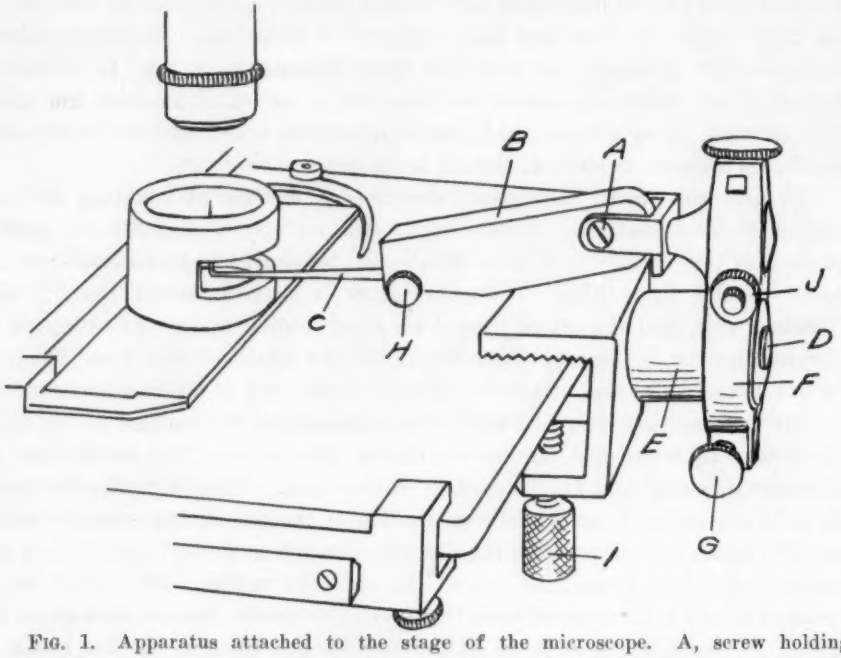

FIG. 1. Apparatus attached to the stage of the microscope. A, screw holding wooden arm in position. B, wooden arm bearing glass needle C. D, metal rod projecting from clamp, over which cylindrical piece of wood E is fitted. F, clamping ring held in place by the screw G. H, set screw holding glass needle in position. I, screw attaching the clamp to the microscope stage. J, screw for raising and lowering the needle point.

of the microscope. The device for raising and lowering the glass needle was obtained from a camera lucida manufactured by the Spencer Lens Co. When used with the camera lucida, this device is required to center the small prism above the ocular of the microscope. By removing the screw A, the prism may be replaced by an arm B made of hard wood, which serves to hold the glass needle C in position. The device was firmly attached to the stage of the microscope by means of a clamp. The clamp shown in the diagram was obtained from a microtome. From the side of the clamp there

projected a metal rod D. A cylindrical piece of wood E was fitted over this rod. The clamping ring F of the camera lucida, which would ordinarily be attached to the draw tube of the microscope, was then passed over the block of wood and secured in the desired position by the screw G.

Glass needles of a suitable size may be made from tubing about 3 mm. in diameter. The tube should be drawn out and bent to a right angle before the point is finished. When a satisfactory point has been obtained, the tube is cut off and the end inserted in the tip of the wooden arm, where it is held in position by means of the set screw H.

A Van Tieghem cell of the size to match the cover-glasses which are to be used may be cut from a block of paraffin. In the front of the cell, there should be an opening large enough to admit the needle and to permit of sufficient up and down movement. In practice, a cell about 25 mm. high has been found to be very satisfactory. The needle is adjusted by slightly releasing screws G and I and moving the apparatus until the point of the needle is exactly in the center of the field. When once this adjustment has been made, the position of the apparatus will require no further altering. All up and down movements of the needle are made by rotating screw J, while lateral movement is secured by shifting the position of the paraffin cell by means of the mechanical stage. Figure 2 shows the apparatus attached to a microscope.

The following procedure was adopted in removing from the promycelium the individual sporidia of *Ustilago zeae* and *Sorosporium reilianum*: A number of petri dishes are fitted with glass Van Tieghem cells as described by Duggar[3] (Fig. 3), each cell being provided with a cover-glass. A filter-paper with holes cut in it for the Van Tieghem cells is placed in each petri dish. When the dishes have been sterilized in a hot-air oven, a drop of sterile 1 per cent malt agar is placed on the lower side of each cover-glass. Using the dry needle method,[4] a single chlamydospore is then placed on each drop of agar. When a spore has germinated and the sporidia have reached full size, the cover-glass is placed on the paraffin cell shown in figure 1. The spore is brought into the center of the field by moving the mechanical stage. By rotating screw J, the needle is moved upward until its point touches the liquid film immediately below the desired sporidium. The needle is lowered slightly, and then, by moving the mechanical stage, the sporidium in the cone of liquid is drawn away until it is some distance from the chlamydospore. After waiting a moment, the needle is lowered, when it will be found that the sporidium has left the agar and has remained

[3] Duggar, B. M. Fungous Diseases of Plants. 508 pp. Ginn and Co. Boston. 1909.

[4] Hanna, W. F. The dry needle method of making monosporous cultures of Hymenomycetes and other fungi. Ann. Bot. 38: 791–795. 1924.

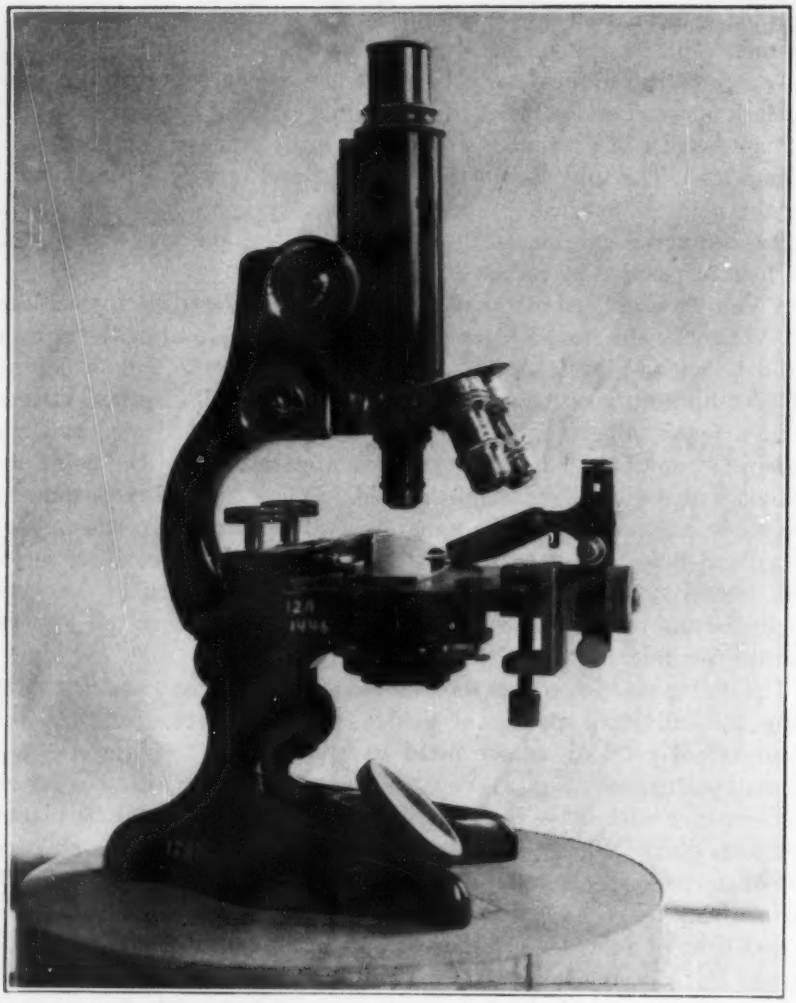

FIG. 2. Microscope equipped with apparatus for isolating single spores.

on the point of the needle. The cover-glass is removed, and another one having a drop of sterile malt agar is placed on the paraffin cell. The point of the needle is then brought upward until the sporidium is deposited on the surface of the agar drop. In this manner all of the sporidia may be removed from the promycelium.

The ease with which the sporidium may be removed from the agar drop is dependent upon the diameter of the needle point and the thickness of the liquid film on the surface of the agar. The primary sporidia of *U. zeae*, when they have reached maturity, are about $8 \times 2\,\mu$, and, in practice, it has

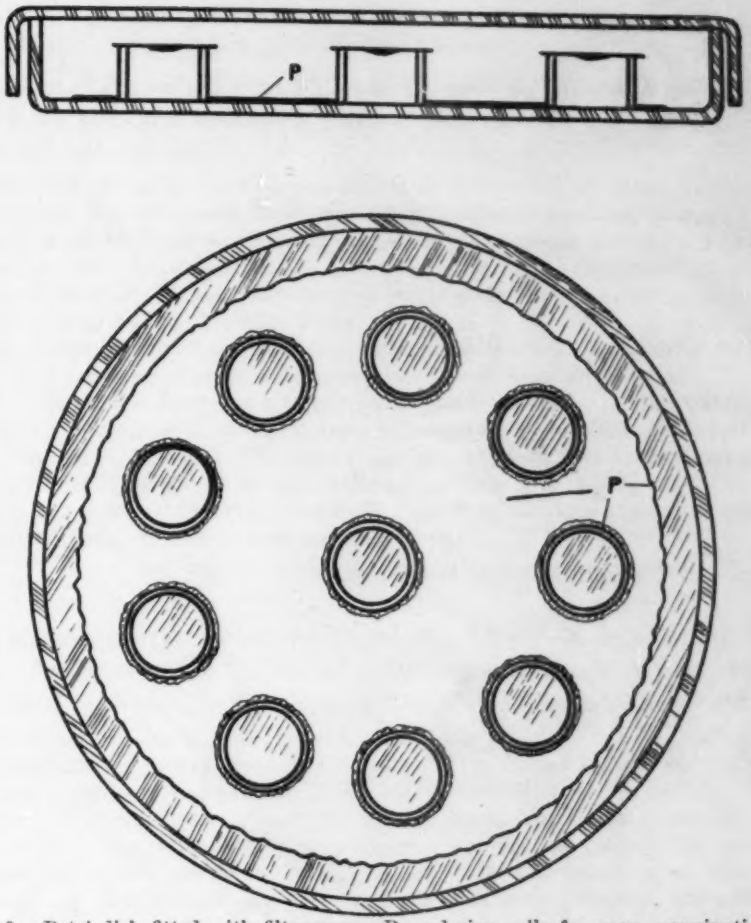

FIG. 3. Petri dish fitted with filter-paper, P, and ring cells for spore germination.

been found that they may be removed readily with a needle having a point about 15 μ in diameter. When the cover-glass is taken from the saturated atmosphere of the petri dish, the film of liquid on the surface of the agar is generally so thick that it is very difficult to remove the sporidium on the point of the needle. However, if the cover-glass is placed on the paraffin cell a few minutes before the work of isolation is to be commenced, the excess moisture will evaporate off and no difficulty will be experienced. If the air of the room is hot and dry, the moisture may evaporate from the drop too rapidly. This difficulty may be overcome by lining the inside of the paraffin cell with a strip of filter-paper and moistening the latter with sterile distilled water.

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BOOK REVIEWS

A List of Japanese Fungi Hitherto Known. By M. Shirai and K. Hara. Third, revised and enlarged, edition. vi + 448 + 14 + 30 + 12 pp. Nov. 1927, Shizuoka, Japan.

The first edition of this book was written in 1905 by Dr. M. Shirai, honorary professor of the Tokyo Imperial University. In 1917 it was revised and enlarged by Professor I. Miyake of the Tokyo Agricultural College. The present publication is the third edition, revised and enlarged by Mr. K. Hara, expert of the Prefectural Agricultural Society of Shizuoka. It is a very laborious and celebrated work, containing about 4,500 species of fungi hitherto known in Japan.

In addition, a list of bacterial plant pathogens known in Japan is also given by Dr. K. Nakata, professor of plant pathology of the Kyûsyû Imperial University.

In this list the fungi are arranged alphabetically according to their Latin names, and some important references in European languages are given, although the host names are given only in Japanese. Therefore it seems to be very useful to the European and American mycologist as well as plant pathologist. This book may be secured from the reviser, Mr. K. Hara, c/o The Agricultural Society of Shizuoka Prefecture, Shizuoka, Japan. The price is three dollars, including postage.

Dr. Y. Nisikado, Ohara Institute, Kurashiki, Japan.

Plantsiektes Hul Oorsaak en Bestryding. By P. A. van der Bijl, M.A., D.Sc., Professor at the University of Stellenbosch, South Africa. 404 pp. Published by Nasionale Pers Beperk. Kaapstad, South Africa. 1928.

Professor van der Bijl's new text-book on plant pathology is written in an easily understandable way, so that not only the South African student but also the South African farmer will be able to profit by it.

The introduction gives a short summary of the history of plant pathology. The book proper is divided into three parts. In the first part are discussed the nature of the plant pathogenes, mainly the fungi; the characteristic structures of the main types of fungi and their terminology; and finally the main orders, families, and genera of fungi. The second part deals with the etiology and control of diseases of plants; and with the fungicides, their composition, usefulness, and application. The third part, which comprises half of the book, is devoted to the more important diseases of economic plants in South Africa. Each disease is treated in a brief way as follows: the name of the disease and the host, the conditions most favorable for the development of the disease, a short description of the fungus, its life history, and the means of control.

A large number of illustrations and references and an adequate index add to the usefulness of the book.

R. Spalteholz, Cornell University, Ithaca, N. Y.

PHYTOPATHOLOGICAL NOTES

A preliminary report[1] *on mechanical transmission of the mosaic of Lilium auratum.*—One of the chief troubles connected with the successful growing of *Lilium auratum* Lindl. in gardens and in greenhouses is a virus disease of the mosaic type. Its symptoms are a marked stunting of the suscept together with a pronounced mottling of the leaves. Frequently, in cases of severe infection, the leaves are twisted and curled as well as mottled and the buds and flowers so distorted as to be valueless. Affected plants rarely survive more than a year or two in the garden.

Attempts were made to transmit this disease mechanically. The plants used were imported as bulbs from Japan and were grown in 6-inch pots in a greenhouse. Only normal, healthy plants which exhibited no mottling were used, and care was taken to keep the greenhouse free from insects. Twenty of these plants were kept as checks during the time the experiments were in progress. All check plants remained healthy.

The first successful transfer was obtained by inarching a healthy and a mosaic plant. The lower leaves were removed, and by means of a razor a small slice was taken from the side of each stem. The cut surfaces were then quickly bound together with raffia and waxed with a mixture of paraffin and petroleum-jelly. Four weeks later the plants had joined sufficiently so that the raffia and paraffin mixture could be removed without any danger of disturbing the union. In a group of four pairs of plants so treated, successful transfers were obtained in two of the cases. Infection first appeared on that side of the healthy plant where the union occurred. The first symptom, appearing eight days after the stems were bound together, was a yellowing of the veins in the basal portions of those leaves which at the beginning of the experiment were about one-half matured. All subsequent leaves which developed were mottled and showed all of the symptoms typical of the disease on this lily. Leaves which were full-grown at the time of inarching remained normal in all respects. Despite the fact that a union occurred, the other two plants in this experiment failed to give any evidence of infection. The experiment was repeated again with four sets of plants. As in the case of the first experiment, two of the healthy plants became mottled and the other two failed to show infection. As far as is known, this is the first time that mosaic in lilies has been experimentally transmitted.

[1] The work here reported was conducted in connection with the Lily Disease Investigation Fellowship established through the cooperation of the Horticultural Society of New York, The Boyce Thompson Institute for Plant Research, The Department of Plant Pathology at Cornell University, and the New York Botanical Garden.

Following these experiments, attempts were made to effect transmission by means of the juice from diseased plants. It was found that the disease is not difficult to transmit mechanically but that it is apparently not so highly infectious as mosaic of tobacco or cucumber. Well-mottled leaves from mosaic plants were removed and ground in a sterile mortar together with sterile distilled water. Two leaves on each of six healthy plants were then inoculated by pricking and scratching with black enamel insect pins which had been dipped in the juice from diseased plants. After eight days, three of the six plants so inoculated became diseased. All of the six checks remained healthy while the plants were under observation. As in the case of the inarched plants the first symptoms appeared in the form of yellowing of the veins in those leaves which were about one-half matured at the time of inoculation. With the same number of plants, this experiment was repeated, and this time successful transfers were obtained with four of the six inoculated plants, all checks remaining healthy. Similar results were obtained in experiments in which about 300 plants were used of this and several other species of the genus *Lilium*. These will be reported upon later.—CARL E. F. GUTERMAN, Boyce Thompson Institute, Yonkers, N. Y.

Oxidizing agents in sulphur to increase fungicidal activity.—Having obtained partial control of the Helminthosporium disease of sugar cane called eye-spot, with sulphur-dust applications, we attempted late in the winter months of 1925–26 to increase the fungicidal activity of the sulphur by the addition of oxidizing agents. These attempts were sufficiently successful to warrant experiments on a much larger scale, which gave very successful results in the winter of 1926–27.[1,2]

The present note is for the comparison of these aforementioned results with interesting results obtained by Bailey and Greaney[3] with sulphur plus an oxidizing agent for stem rust of wheat in Manitoba. These investigators report that slightly better control of rust was obtained by using oxidized sulphur than by using unoxidized colloidal sulphur. In their experiments two plots received each treatment, and the percentages of rust infection apparently were determined by a visual comparison of the plots by persons with no knowledge of the treatments that had been given so that they could not show prejudice for or against any treatment.

[1] LEE, H. ATHERTON and J. P. MARTIN. The development of more effective dust fungicides by adding oxidizing agents to sulphur. Science, **66**: 178. 1927.

[2] ————, and ————. More effective dust fungicides by the use of oxidizing agents with sulphur. Industr. and Engineering Chem., **20**: 23. 1928.

[3] BAILEY, D. L. and F. J. GREANEY. Dusting with sulphur for the control of leaf and stem rust of wheat in Manitoba. Sci. Agr., **8**: 409. 1928.

In this connection we wish to establish in an abstract way a point as follows: In our experiments on sugar cane the degree of infection under the various fungicidal treatments was usually determined by counting the number of infections on from 20 to 40 leaves in a plot, in series of plots with from 6 to 8 or usually 10 replications of the plots for each treatment. These infection counts were then averaged for each leaf, giving us a very good measure of the degree of infection under each fungicidal treatment. The broad leaves of sugar cane, with new leaves forming with fairly uniform regularity, were notably advantageous for such a quantitative determination of the degree of infection.

In this work it was found that visual observation methods of determining the degree of infection were far from being as exact or informative as the quantitative measure of infection obtained by infection counts. We and our associates arrived at the conclusion that we could not observe a difference in the degree of infection in the plots unless the difference exceeded 33 per cent of the amounts of infection. In other words, when treatments gave differences of infection which were readily observable by mere general observation, the differences in degree of infection by actual count usually exceeded 33 per cent.

We therefore desire to call attention to the favorable results which oxidized sulphur gave in the control of eye-spot disease of sugar cane, and to the favorable results which Bailey and Greaney later obtained with stem rust of wheat. We feel that oxidized sulphur will be sufficiently more efficient than the unoxidized sulphur to put dusting operations in more even competition with liquid sprays for prevention of other crop diseases, with the added advantage which dusting operations have over liquid sprays in economy, mobility, and quickness of application. We feel that the control of some of the diseases of other crops could be made more effective and economical by the use of these oxidized sulphur mixtures and wish to ask that other investigators give them a trial.—H. ATHERTON LEE and J. P. MARTIN, Experiment Station of the Hawaiian Sugar Planters' Association, Honolulu, T. H.

Forced Ventilation as a Means of Controlling Tomato Cladosporium and Septoria in Hotbeds.[1]—Tomato plants grown for 45 days in a manure-heated hotbed and inoculated at two different times[2] with *Cladosporium fulvum* and *Septoria lycopersici*, but kept dry both day and night by means of an electric heater and electric fan did not become infected with either

[1] Approved for publication by the Director of the Purdue Agricultural Experiment Station.

[2] Inoculum furnished by Dr. M. W. Gardner, who also confirmed the disease identification.

fungus. Plants grown in an adjacent control bed and similarly inoculated were badly infected with both fungi. Inoculated plants in a third adjacent hotbed were kept free from both diseases by applications of copper lime dust (Niagara D. 25) at weekly intervals.

The use of forced ventilation for controlling leaf spot diseases is of great interest to plant growers and vegetable and flower forcers. It provides an added control measure which helps to insure the control of these diseases under forcing structures.

The circulation of heated air was obtained by drawing air over a 250-watt electric heater by means of a 12-inch electric fan. The heated air with its higher moisture-holding capacity provided a medium for the removal of water from the leaves, including guttation water, even though the relative humidity of the outside air was near the saturation point. The fan, although entirely too large, brought this drier air in contact with all the foliage. On windy and extremely cold nights the heater was effective without the use of the fan. The heater thus served a dual purpose on nights when added heat was most needed. The arrangement of the heater and fan is shown in figure 1.

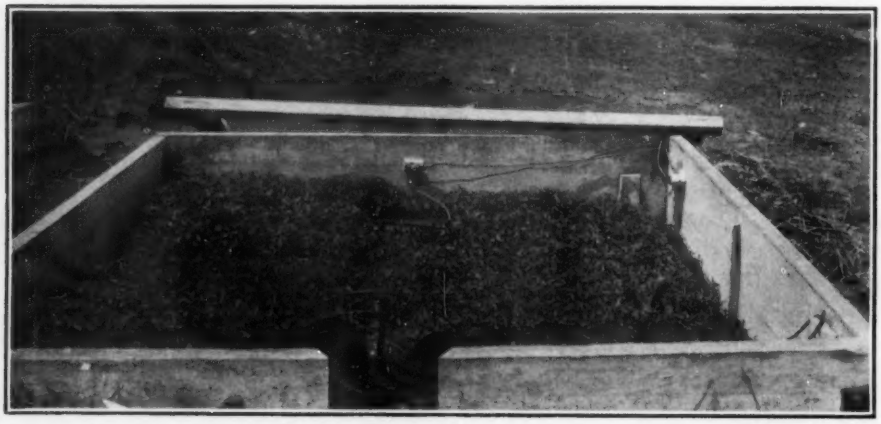

FIG. 1.—Location of electric heater at far end of bed and electric fan used for heating and circulating air in manure-heated hotbed.

Because cold air was being introduced constantly from the outside, the day temperatures in the bed supplied with forced ventilation were 8–10° F. lower than those of the control bed and 2–3° F. lower than those in the bed in which the plants were dusted. The average daily temperature at 11 a. m. was 73° F. in the control bed, in the dusted bed 66° F., and 64° F. in the bed receiving the forced ventilation. This lower temperature is

largely responsible for the production of stockier plants in the bed provided with forced ventilation.

The temperature difference may also be responsible for some of the difference in disease prevalence, although it is our opinion that freedom from disease in the forced ventilated bed is due largely to the lack of moisture on the foliage of the plants. At any rate the conditions existing in the control bed closely approximate those found in many commercial beds.

Although these results are not sufficient to warrant wholesale installation of electric heaters and electric fans in our forcing structures, it does seem that precautions should be taken to provide more ventilation for the purpose of keeping the plant foliage dry and perhaps to keep the temperatures lower. Similar results were obtained by Newhall[3] under greenhouse conditions. Replacing the sash during rainy periods for the purpose of keeping plant foliage dry is also suggested. The use of fungicides in addition to the forced ventilation should of course be continued in order to make the control as nearly complete as possible.—E. C. STAIR, H. D. BROWN, T. E. HIENTON, Purdue Agricultural Experiment Station.

Permanent mounts of white pine blister rust aecia.—The delicate aecia of *Cronartium ribicola* on pine branches fade and disintegrate in a short time to such an extent that they are soon useless for demonstration purposes. The following method enables one to preserve them in a state closely approximating their normal condition. When the blisters are mature and about to burst, they are treated with a staining solution consisting of about equal parts of orange G and eosin in 95 per cent alcohol. A bit of absorbent cotton twisted around a match or a camel's hair brush may be used to apply the stain. Each blister should be thoroughly impregnated. When the alcohol has evaporated, after about an hour, the same impregnation process is repeated with a 10 per cent solution of paraffin wax in xylol or other similar solvent. Upon drying out, the blisters are surprisingly firm and have a permanent color very like the natural tint.—W. A. MCCUBBIN, Pennsylvania Bureau of Plant Industry, Harrisburg, Pa.

Eriksson prizes.—The Committee beg to announce that two prizes are hereby offered for the two best memoirs, giving an account of new and original work on the two following subjects respectively: (1) Investigations on rust (Uredineae) diseases of cereals (wheat, oats, barley or rye); (2) Investigations on the rôle played by insects or other invertebrates in the transmission or initiation of virus disease in plants.

[3] Newhall, A. G., Fan ventilation and greenhouse tomato leaf mold. American Produce Grower 3: 5. 1928.

The value of each prize will be 1,000 Swedish crowns. Competitors may be of any nationality. Three typewritten copies of each memoir must be submitted, written in English, French, or German. Memoirs must reach the Secretary of the Committee, Mr. T. A. C. Schoevers, Wageningen, Holland, on or before May 1, 1930.

The author's name must not appear on the memoir itself, but each memoir must be marked with a pseudonym or a motto and the full name and address of the author must accompany the memoir, being enclosed in a sealed envelope bearing on its outside the same pseudonym or motto as is given on the memoir.

The adjudication of the rust prize will rest with a jury, consisting of Professor Dr. Jacob Eriksson, Professor Dr. E. C. Stakman, and Professor M. Et. Foëx. The jury for the virus prize will be announced as soon as possible. The decisions of these juries will be final, and will be announced at the Fifth International Botanical Conference, to be held in Cambridge (England), August 16–30, 1930. The copyright of the prize memoirs will become the property of the committee, who will endeavor to secure publication of them in a suitable periodical or in some other way. Other memoirs will be returned to authors.

The committee reserve the right to withhold the prizes should none of the memoirs submitted be deemed of sufficient merit by the respective juries. Further particulars, if required, may be obtained on application to the Secretary, at the above-mentioned address.

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